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RELATED APPLICATIONS

This application is a continuation-in-part of U.S. application Serial No. ___________, filed January 16, 2001 (Attorney Docket No. 454313-3154.1); and, this application claims priority from U.S. Provisional application Serial No.60/193,126, filed 30 March 2000, and French application No. 00 00798, filed January 21, 2000. Mention is also made of U.S. applications Serial Nos. 09/232,468, 09/232,469 and 09/232,279, each filed January 15, 1999. Each of the foregoing applications, and all documents cited therein or during their prosecution ("appln cited documents") and all documents cited or referenced in the appln cited documents, and all documents cited or referenced herein ("herein cited documents"), together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein, are hereby incorporated herein by reference.

The present invention relates to improved DNA vaccines or immunogenic or immunological compositions for farm animals, in particular bovines and porcines.

The use of deoxyribonucleic acid (DNA) molecules for vaccination has been known since the beginning of the 1990s (Wolf *et al.* Science 1990. **247.** 1465-1468). This vaccination technique induces cellular and humoral immunity after *in vivo* transfection of cells of the subject to be vaccinated with DNA or RNA molecules encoding immunologically active proteins.

A DNA vaccine or immunogenic or immunological composition is composed of at least one plasmid which may be expressed by the cellular machinery of the subject to be vaccinated or inoculated and of a pharmaceutically acceptable vehicle or excipient. The nucleotide sequence of

this plasmid encodes, inter alia, one or more immunogens, such as proteins or glycoproteins capable of inducing, in the subject to be vaccinated or inoculated, a cellular immune response (mobilization of the T lymphocytes) and a humoral immune response (stimulation of the production of antibodies specifically directed against the immunogen) (Davis H.L. Current Opinion Biotech. 1997. **8**. 635-640).

All the immunogens derived from a pathogen are not antigens which are naturally sufficiently effective for inducing an optimum or protective immune response in the animal to be vaccinated or inoculated. It is therefore necessary to improve the immune response.

Various routes of administration of the DNA vaccine have been proposed (intraperitoneal, intravenous, intramuscular, subcutaneous, intradermal, mucosal, and the like). Various means of administration have also been proposed, in particular gold particles coated with DNA and projected so as to penetrate into the cells of the skin of the subject to be vaccinated (Tang *et al.* Nature 1992. **356.** 152-154) and the liquid jet injectors which make it possible to transfect both skin cells and cells of the underlying tissues (Furth *et al.* Analytical Bioch. 1992. **205**. 365-368).

Chemical compounds have been used for the *in vitro* transfection of DNA:

A/ - cationic lipids.

The cationic lipids are themselves divided into four subgroups.

1) The cationic lipids containing quaternary ammonium salts, such as for example DOTMA (dioleoyl-oxypropyltrimethylammonium, produced by Gibco under the name Lipofectine), DOTAP (trimethyl-2,3-(octadec-

9-eneoyloxy)-1-propaneammonium; Gregoriadis *et al.* FEBS Letters 1997. **402.** 107-110), DMRIE (N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propaneammonium; WO-A-9634109), DLRIE (N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(dodecyloxy)-1-propaneammonium; Felgner *et al.* Ann. N Y Acad. Sci. 1995. **772.** 126-139).

These cationic lipids containing quaternary ammonium salts may be combined or otherwise with an additional neutral lipid, such as DOPC (dioleoyl-phosphatidylcholine) or DOPE (dioleoylphosphatidyl-ethanolamine) (J.P. Behr, Bioconjugate Chemistry 1994. **5.** 382-389).

2) The lipoamines, such as for example DOGS (dioctadecylamidoglycylspermine, produced by Promega under the name Transfectam; Abdallah *et al.* Biol. Cell. 1995. **85.** 1-7), DC-Chol (dimethylaminoethane-carbamoyl-cholesterol; Gao and Huang, Biochem. Biophys. Res. Commun. 1991. **179.** 280-285), BGSC (bis-guanidine-spermidine-cholesterol), BGTC (bis-guanidine-trencholesterol) (Vigneron *et al.* Proc. Natl. Acad. Sci. USA 1996. **93.** 9682-9686).

3) The cationic lipids containing quaternary ammonium salts and lipoamines, such as for example DOSPA (N,N-dimethyl-N-(2-(sperminecarboxamido)ethyl)-2,3-bis(dioleoyloxy)-1-propaneimidium pentahydrochloride, marketed by Gibco under the name LipofectAmine®; Hawley-Nelson *et al.* Focus 1993. **15.** 73-79), GAP-DLRIE (N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(dodecyloxy)-1-propaneammonium; Wheeler *et al.* Proc. Natl. Acad. Sci. USA 1996. **93.** 11454-11459; Norman *et al.* Vaccine 1997. **15.** 801-803).

4) The lipids containing amidine salts, such as for example ADPDE, ADODE (Ruysschaert *et al.* Biochem. Biophys. Res. Commun. 1994. **203**. 1622-1628).

B/ - the polymers, such as for example SuperFect™ (molecules of activated dendrimers, produced by Qiagen; Xu *et al.* Mol. Genet. Metab. 1998. **64**. 193-197), and

C/ - the biochemical agents, such as for example toxins, in particular cholera toxins.

Some of these compounds have also been used in the formulation of DNA vaccines with more than mitigated results. Knowledge in the field of *in vitro* transfection is not transposable to DNA vaccination where the final objective is to ensure an optimal and advantageously protective immune reaction. Negative effects on the induction of an effective immune protection have even been observed with compounds known to promote transfection *in vitro*. Some formulation chemical compounds are toxic at high doses for the transfected cells.

In the work by Etchart (Etchart *et al.* J. Gen. Virol. 1997. **78**. 1577-1580), the use of DOTAP did not have an adjuvant effect during the administration of the DNA vaccine by the intranasal route, whereas it had an adjuvant effect by the oral route. DOTAP has also been used in DNA vaccines encoding the influenza virus hemagglutinin (HA) on the mouse model which were administered by the intranasal route (Ban *et al.* Vaccine 1997. **15**. 811-813), but the addition of DOTAP inhibited the immune response. The use of DC-Chol or of DOTAP/DOPE in DNA vaccines encoding the hepatitis B virus surface protein (S) on the mouse model which were administered by the intramuscular route made it possible to increase the antibody response, whereas the use of Lipofectine (or DOTMA) did not increase this response

(Gregoriadis *et al.* FEBS Letters 1997. **402.** 107-110). DC-Chol/DOPE has also been used in DNA vaccines against the human immunodeficiency virus (HIV, Env protein) on the mouse model, whose administration by the intramuscular route induced a more effective immune response, whereas the administration by the subcutaneous or intradermal route did not increase it (Ishii *et al.* AIDS Res. Hum. Retro. 1997. **13.** 1421-1428).

The addition of certain cytokines, in particular interleukins or interferons, can make it possible to enhance the immune response induced in particular by DNA vaccines. Each cytokine triggers a reaction which is specific to it and orients the immune response to a greater or lesser degree towards a cellular response or towards a humoral response (Pasquini *et al.* Immunol. Cell. Biol. 1997. **75.** 397-401; Kim *et al.* J. Interferon Cytokine Res. 1999. **19.** 77-84). The adjuvant effects of a cytokine obtained from a given species are not necessarily the same if the immune context varies, in particular if this cytokine is administered to another species, therefore in a heterologous immune system. The addition of cytokine may also have no adjuvant effect, or may even result in a reversal of the effect sought, that is to say a reduction or an inhibition of the immune response. Thus, a DNA vaccine encoding a single chain of an immunoglobulin fused with GM-CSF does not increase the immune response, whereas direct administration of this fusion protein to mice is effective, in the same way as is the administration of a fusion protein consisting of Fv and of the cytokine IL-1beta or the administration of a DNA vaccine encoding the latter fusion protein (Hakim *et al.* J. Immunol. 1996. **157.** 5503-5511). The use of plasmids co-expressing the cytokine

IL-2 and the hepatitis B virus envelope protein in a fused or nonfused conformation results in an increase in the humoral and cellular immune responses (Chow *et al.* J. Virol. 1997. **71.** 169-78). However, the use of a bicistronic plasmid encoding the human acquired immunodeficiency virus (HIV-1) glycoprotein gp120 and the cytokine IL-2 induced a lower specific anti-gp120 immune response than that obtained by the use of a monocistronic plasmid encoding only gp120 (Barouch *et al.* J. Immunol 1998. **161.** 1875-1882). The co-injection, into mice, of two expression vectors, one coding for the rabies virus G glycoprotein, the other for murine GM-CSF stimulates the activity of the B and T lymphocytes, whereas the co-injection with a plasmid encoding gamma-interferon (in place of murine GM-CSF) results in a decrease in the immune response (Xiang *et al.* Immunity 1995. **2.** 129-135).

Certain modifications in the antigens, such as deletions of part of the nucleotide sequence encoding the antigen, insertions of a DNA fragment into the nucleotide sequence encoding the antigen or into non-translated regions upstream or downstream, can also enhance the efficacy of DNA vaccines, in particular by enhancing the level of expression of the antigen or its presentation.

However, in practice, manipulations on the nucleotide sequence encoding the antigen may bring about a reduction or loss of the initial immunological activity. Thus, the deletion of the transmembrane domain from the gene encoding the rabies virus G antigen reduced the level of protection induced in the mouse model after administration by the intramuscular route of a DNA vaccine encoding this modified antigen (Xiang *et al.* Virol. 1995. **209.** 569). The deletion of the transmembrane domain from

the gene encoding the bovine herpesvirus (BHV) gD glycoprotein did not make it possible to increase the antibody response and induced only a partial protection in bovines vaccinated by the intramuscular route (van Drunen Little-van den Hurk *et al.* J. Gen. Virol. 1998. **79.** 831-839). The humoral and cellular immune responses and the protection conferred are identical in guinea pigs challenged after having been immunized with the aid of either a DNA vaccine encoding the Ebola virus GP glycoprotein, or of a DNA vaccine encoding this GP glycoprotein but in a secreted form (Xu *et al.* Nature Medicine 1998. **4.** 37-42).

The insertion of the signal sequence of the human tissue plasminogen activator (tPA) into the gene encoding the malaria Pf332 antigen did not make it possible to increase the antibody response in mice vaccinated by the intramuscular route (Haddad *et al.* FEMS 1997. **18.** 193-202). The addition, in phase, of a tPA sequence to the gene encoding the murine rotavirus VP7 antigen also did not make it possible to increase the antibody response in mice vaccinated by the intradermal route, whereas the fusion protein consisting of the VP4 antigen and tPA allowed this increase, but without inducing an effective protection (Choi *et al.* Virology 1998. **250.** 230-240).

The modifications carried out on the nucleotide sequence of one antigen cannot in general be directly transposed to another antigen, because antigens do not always have the same structural arrangements.

The applicant has as objective the enhancement of the efficacy of DNA vaccination or immunization. Its objective is in particular to obtain a better immune response and advantageously effective protection in farm

animals, preferably bovines and porcines, by DNA vaccinations or immunizations.

The applicant has as objective the production of improved DNA vaccines or immunogenic or immunological compositions which induce an improved effect an advantageously an effective and/or protective immune response against the bovine herpesvirus type 1 (BHV-1) also called infectious bovine rhinotrachitis (IBR), the bovine respiratory syncitial virus (BRSV), the mucosal disease virus or bovine pestivirus type 1 or type 2 (bovine viral diarrhea virus or BVDV-1 and BVDV-2), the parainfluenza virus type 3 (bPI-3) in bovines.

The applicant has as an objective the production of improved DNA vaccines or immunogenic compositions or immunological compositions which induce an improved and advantageously effective and/or protective immune response comprising at least one valency selected from the group consisting of porcine herpesvirus or Aujeszky's disease (pseudorabies virus or PRV), the porcine reproductive respiratory syndrome virus (or PRRSV), the swine influenza virus (or SIV), the conventional hog cholera virus (or HCV), parvoviruses in porcines.

The applicant also has as objective the production of improved DNA vaccines or immunogenic compositions or immunological compositions which make it possible to obtain an improved or advantageously effective and/or protective immune protection in bovines, comprising at least one valency selected from the group consisting of the BHV-1, BRSV, BVDV, bPI-3 and rabies viruses.

The subject of the invention is improved DNA vaccines or immunogenic or immunological compositions which make it possible to obtain an improved immunological or

immunogenic effect, such as effective protection, against at least one pathogen which infects farm animals, such as bovines and porcines. The DNA vaccine or immunogenic or immunological composition is improved: either by its formulation, or by the addition of GM-CSF, or by the optimization of the antigen(s), or by combinations of these solutions.

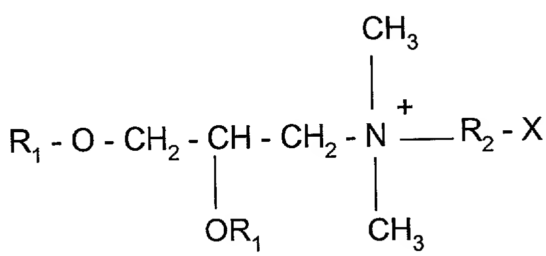
Preferably, the DNA vaccine or immunogenic or immunological composition is improved by its formulation, and optionally either by the addition of GM-CSF, or by the optimization of the antigen(s), or finally by the addition of GM-CSF and by the optimization of the antigen(s).

By definition, the DNA vaccine or immunogenic or immunological composition comprises, as active ingredient, a plasmid encoding and expressing a gene or gene fragment e.g. epitope. The term plasmid covers a DNA transcription unit comprising a polynucleotide sequence comprising the sequence of the gene to be expressed and the elements necessary for its expression *in vivo*. The circular plasmid form, supercoiled or otherwise, is preferred. The linear form also falls within the scope of this invention.

Each plasmid comprises a promoter capable of ensuring, in the host cells, the expression of the gene inserted under its control. It is in general a strong eukaryotic promoter and in particular a cytomegalovirus early promoter CMV-IE, of human or murine origin, or optionally of other origin such as rat or guinea pig. More generally, the promoter is either of viral origin or of cellular origin. As a viral promoter other than CMV-IE, there may be mentioned the SV40 virus early or late promoter or the Rous Sarcoma virus LTR promoter. It may also be a promoter the virus from which the gene is

derived, for example the promoter specific to the gene. As cellular promoter, there may be mentioned the promoter of a cytoskeleton gene, such as for example the desmin promoter, or alternatively the actin promoter. When several genes are present in the same plasmid, they may be provided in the same transcription unit or in several different units.

According to a first mode, the DNA vaccines or immunogenic or immunological compositions according to the invention are formulated by adding, as adjuvant, cationic lipids containing a quaternary ammonium salt of formula:



in which R_1 is a saturated or unsaturated linear aliphatic radical having 12 to 18 carbon atoms, R_2 is another aliphatic radical containing 2 or 3 carbon atoms, and X a hydroxyl or amine group.

Preferably, this is DMRIE (N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanammonium ; WO-A-9634109), preferably combined with a neutral lipid, in particular, DOPE (dioleoyl-phosphatidyl-ethanolamine), to form DMRIE-DOPE.

The subject of the present invention is therefore a DNA vaccine or immunogenic or immunological composition against at least one pathogen affecting farm animals, such as bovines or porcines, comprising at least one plasmid containing at least one nucleotide sequence encoding an

immunogen of a pathogen of the animal species considered, under conditions allowing the *in vivo* expression of this sequence, and a cationic lipid containing a quaternary ammonium salt, in particular DMRIE, preferably combined with DOPE.

Preferably, the recombinant vector is mixed with this adjuvant immediately before use and it is preferable, before its administration to the animal, to allow the mixture thus prepared to form a complex, for example for a period ranging from 10 to 60 minutes, in particular of the order of 30 minutes.

When DOPE is present, the DMRIE:DOPE molar ratio preferably ranges from 95:5 to 5:95, and is more particularly 1:1.

The plasmid:DMRIE or DMRIE-DOPE adjuvant weight ratio may range in particular from 50:1 to 1:10, in particular from 10:1 to 1:5, preferably from 1:1 to 1:2.

According to a second mode, GM-CSF (granulocyte macrophage-colony stimulating factor; Clark S.C. *et al.* Science 1987. **230**. 1229; Grant S.M. *et al.* Drugs 1992. **53**. 516) is added to the vaccines or immunogenic or immunological compositions according to the invention; this may be carried out by incorporating GM-CSF protein directly into the vaccinal or immunogenic or immunological composition or preferably by inserting the nucleotide sequence encoding GM-CSF into an expression vector under conditions allowing its expression *in vivo*. As expression vector, the use of a plasmid, e.g. the plasmid containing the nucleotide sequence encoding the antigen(s) of interest or another plasmid, is preferred. The choice of GM-CSF is preferably made according to the animal species to be

vaccinated; thus, for bovines, bovine GM-CSF is used; for pigs, it is porcine GM-CSF.

According to a third mode, the nucleotide sequence(s) encoding the immunogen are in an optimized form. Optimization is understood to mean any modification of the nucleotide sequence, in particular which manifests itself at least by a higher level of expression of this nucleotide sequence, and/or by an increase in the stability of the messenger RNA encoding this antigen, and/or by the triggered secretion of this antigen into the extracellular medium, and having as direct or indirect consequence an increase in the immune response induced.

In the present invention, the optimization of the antigen of interest preferably consists in the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain of the antigen of interest (deletion is understood to mean the complete deletion or a partial deletion sufficient for the transmembrane domain to no longer, or no longer substantially, be functional), and/or in the addition, in frame, of a nucleotide sequence encoding the tPA (Montgomery *et al.* Cell. Mol. Biol. 1997. **43**. 285-292; Harris *et al.* Mol. Biol. Med 1986. **3**. 279-292) signal, and/or in the insertion of a stabilizing intron upstream of the gene to be expressed. The deletion of the DNA fragment encoding the transmembrane domain of the antigen of interest promotes the secretion, into the extracellular medium, of the antigens thus truncated and thus increases the possibilities of their coming into contact with the cells of the immune system. The insertion of the nucleotide sequence encoding the tPA signal facilitates the translatability of the messenger RNA to which the tPA signal is joined, and thus increases the

level of expression of this messenger RNA and therefore the production of antigens. The tPA signal also plays a role in the secretion of the antigen synthesized.

Other nucleotide sequences encoding signal peptides may be used, in particular those for the signal peptide of melittin obtained from bees (Sisk W.P. *et al.*, 1994, J. Virol., **68**, 766-775).

The insertion of a stabilizing intron into the gene encoding the antigen of interest avoids the aberrant splicings of its messenger RNA and maintains the physical integrity of the latter.

Preferably, the tPA signal is of human origin. The nucleotide sequence of the human tPA signal is accessible from the GenBank database under the accession number NM_000930. Preferably, the intron is intron II of the rabbit beta-globin gene (van Ooyen *et al.* Science 1979. **206**. 337-344), whose nucleotide sequence is accessible from the GenBank database under the accession number V00882 and designated by a reference under intron No. 2.

The subject of the present invention is an improved DNA vaccine or immunogenic or immunological capable of inducing an improved immune response, advantageously an effective and/or protective immune response in bovines against infectious bovine rhinotrachitis (IBR).

The virus responsible for infectious bovine rhinotrachitis is a bovine herpesvirus type 1 (BHV-1), a member of the *Alphaherpesvirinae* family (Babiuk L.A. *et al.*, 1996, Vet. Microbiol., **53**, 31-42). Nucleotide sequences encoding the glycoproteins gB, gC and gD are known and are accessible from the GenBank database under the accession number AJ004801.

According to the invention, the DNA vaccine or immunogenic or immunological composition against IBR is preferably improved by its formulation with an adjuvant according to the invention, in particular DMRIE, preferably DMRIE-DOPE. Optionally, this may be combined either with the addition of bovine GM-CSF (Maliszewski *et al.*, Molec. Immunol., 1988, **25**, 843-850), or the optimization of at least one IBR antigen, or finally the addition of bovine GM-CSF and the optimization of at least one IBR antigen.

A nucleotide sequence encoding bovine GM-CSF is accessible from the GenBank database under the accession number U22385.

The addition of bovine GM-CSF may be carried out by the incorporation of the bovine GM-CSF polypeptide into the vaccinal or immunogenic or immunological composition or preferably by the insertion of the nucleotide sequence encoding the bovine GM-CSF into an *in vivo* expression vector, preferably a plasmid. Preferably, the nucleotide sequence encoding bovine GM-CSF is inserted into a second expression plasmid (e.g. pLF1032 Example 13), different from that (or those) into which the gene(s) encoding the IBR antigen(s) is(are) inserted.

The optimization of the antigens derived from IBR is carried out by substitution, by a "signal" sequence, in particular that of the tPA signal of human origin (GenBank accession number NM_000930), of the sequence of the signal peptide of the glycoprotein gB and/or of the glycoprotein gC and/or of the glycoprotein gD, and/or by the deletion of the DNA fragment encoding the transmembrane domain of gB and/or of gC and/or of gD. The deletion of the DNA fragment encoding the transmembrane domain of one of these glycoproteins is preferably accompanied by the contiguous

C-terminal part (cytoplasmic portion of the glycoprotein). The DNA vaccine or immunogenic or immunological composition against IBR according to the invention can therefore encode and express a single optimized IBR antigen (gB, gC or gD) or two of them or all three, that is to say optimized gB, optimized gC and optimized gD.

Nucleotide sequences encoding the BHV-1 antigens which can be used in the present invention and various constructs of expression vectors are given in the accompanying examples and in FR-A1-2751229, in particular in Examples 7 and 8, and in Figures 3 and 4.

Preferably, according to the invention, the DNA vaccine or immunogenic or immunological composition against BHV-1 is formulated with DMRIE-DOPE, and is composed of an expression plasmid (e.g. pPB281, Example 3.1.2) encoding the BHV-1 gB antigen optimized by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain and the contiguous C-terminal part, of a second expression plasmid (e.g. pPB292, Example 3.2.2) encoding the BHV-1 gC antigen optimized by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain and the contiguous C-terminal part, and of a third expression plasmid (e.g. pPB284, Example 3.3.2) encoding the BHV-1 gD antigen optimized by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain and the contiguous C-terminal part.

In general, and not only for BHV-1, the C-terminal part contiguous to the sequence encoding the transmembrane domain may be conserved. It is however often easier to delete it at the same time as the sequence encoding the transmembrane domain.

The subject of the present invention is also an improved DNA vaccine or immunogenic or immunological composition capable of inducing an improved or advantageously effective and/or protective immune response in bovines against the bovine respiratory syncitial virus (BRSV).

The BRSV virus is a Paramyxovirus, also a member of the *Paramyxoviridae* family (Baker *et al.*, Vet. Clin. North Am. Food Anim. Pract., 1997, **13**, 425-454). Nucleotide sequences encoding the F protein and the G glycoprotein are known and accessible from the GenBank database respectively under the accession number Y17970 and U33539.

The DNA vaccine or immunogenic or immunological composition against BRSV is preferably formulated with an adjuvant according to the invention, in particular DMRIE, preferably DMRIE-DOPE. This may be optionally combined with either the addition of bovine GM-CSF, or the optimization of at least one BRSV antigen, or finally the addition of bovine GM-CSF and the optimization of at least one BRSV antigen.

The addition of bovine GM-CSF may be carried out as is described for BHV-1.

The optimization of the antigens derived from BRSV is carried out by substitution, by a "signal" sequence, in particular that of the tPA of human origin, of the signal sequence of the F protein of BRSV and/or of the G envelope glycoprotein of BRSV, and/or by the deletion of the DNA fragment encoding the transmembrane domain of F and/or of G. The deletion of the DNA fragment encoding the transmembrane domain of one of these proteins is preferably accompanied by the contiguous C-terminal part. The DNA vaccine or immunological or immunogenic composition against

BRSV according to the invention can therefore encode and express a single optimized BRSV antigen (F or G) or both (F and G).

Nucleotide sequences encoding the BRSV antigens which can be used in the present invention and various expression vector constructs are given in the accompanying examples and in FR-A1-2751229, in particular in Examples 9 and 10, and in Figures 5 and 6.

Preferably, according to the invention, the DNA vaccine or immunogenic or immunological composition against BRSV is formulated with DMRIE-DOPE, and is composed of an expression plasmid (e.g. pSB114 Example 4.1.3) encoding the F antigen of BRSV optimized by the insertion of the signal sequence of the human tPA in place of the signal sequence of F, by the deletion of the fragment of the nucleotide sequence of F encoding the transmembrane domain and the contiguous C-terminal part, and of a second expression plasmid (e.g. pSB110 Example 4.2.2) encoding the G antigen of BRSV optimized by the insertion of the signal sequence of the human tPA in place of the signal sequence of G, by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain of G and the contiguous C-terminal part.

The subject of the present invention is also an improved DNA vaccine or immunogenic or immunological composition capable of inducing an improved or advantageously effective and/or protective immune response in bovines against the BVDV virus.

The BVDV virus is a pestivirus of the *Flaviviridae* family. It is universally distributed in bovine populations and manifests itself by fetal malformations, abortions or

clinical respiratory (mucosal disease) and enteric (bovine viral diarrhea) symptoms.

The BVDV viruses are distinguishable by the seriousness of the clinical signs and two groups have been formed, the BVDVs type 1 (inapparent or mild clinical signs) and those of type 2 (acute clinical signs, hemorrhage, high morbidity, high mortality) (Dean H.J. and Leyh R., 1999, Vaccine, **17**, 1117-1124).

When a BVDV virus type is not clearly specified, this virus is understood to be type 1 or type 2.

The BVDV virus is an enveloped single-stranded RNA virus composed of a single gene encoding a polyprotein which, after cleavage, gives several well-individualized proteins, in particular the E0 protein (gp48) and the E2 protein (gp53) (Vassilev V.B. *et al.*, 1997, J. Virol., **71**, 471-478).

Nucleotide sequences encoding the E0-E2 polyproteins are known and accessible from the GenBank database under the accession number M96687 for BVDV-1 and AF145967 for BVDV-2.

The DNA vaccine or immunogenic or immunological composition against BVDV is preferably formulated with an adjuvant according to the invention, in particular DMRIE, preferably DMRIE-DOPE. This may be optionally combined with either the addition of bovine GM-CSF, or the optimization of at least one BVDV antigen, or finally the addition of bovine GM-CSF and the optimization of at least one BVDV antigen.

The addition of bovine GM-CSF may be carried out as is described for BHV-1.

The optimization of the antigens derived from BVDV is carried out by the addition of a "signal" sequence, in

particular that of the tPA of human origin, upstream of the nucleotide sequence encoding the E0 protein of BVDV and/or of the E2 protein of BVDV, and/or by the deletion of the DNA fragment encoding the transmembrane domain of E2, and/or by the insertion of an intron, in particular intron II of the rabbit beta-globin gene upstream of the nucleotide sequence encoding E0 and/or E2. The DNA vaccine or immunogenic or immunological composition against BVDV according to the invention may therefore encode and express a single optimized BVDV antigen (E0 or E2) or both (E0 and E2).

Nucleotide sequences encoding the BVDV antigens which can be used in the present invention and various constructs of expression vectors are given in the accompanying examples and in FR-A1-2751229, in particular in Example 13, and in Figure 9.

Preferably, according to the invention, the DNA vaccine or immunogenic or immunological composition against BVDV is formulated with DMRIE-DOPE, and is composed of an expression plasmid (e.g. pLF1029 Example 5.1.2, pLF1031 Example 6.2.2) encoding the E0 antigen of BVDV optimized by the insertion of the signal sequence of the human tPA upstream of E0 and by the insertion of intron II of the rabbit beta-globin gene upstream of E0, and of a second expression plasmid (e.g. pLF1021 Example 5.2.2, pLF1023 Example 6.1.2) encoding the E2 antigen of BVDV optimized by the insertion of the signal sequence of the human tPA upstream of E2, by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain of E2 and the contiguous C-terminal part and by the insertion of intron II of the rabbit beta-globin gene upstream of E2.

A mixture of plasmids can be advantageously produced. The mixture may comprise at least two expression plasmids, each expressing a different immunogen (E0 or E2) and/or obtained from a different type of BVDV (BVDV-1 or BVDV-2). In particular, a mixture made of four plasmids expressing BVDV-1 E0, BVDV-1 E2, BVDV-2 E0 and BVDV-2 E2.

The subject of the present invention is also an improved DNA vaccine or immunological or immunogenic composition capable of inducing an improved or advantageously effective and/or protective immune response in bovines against the parainfluenza virus type 3 (bPI-3).

The bPI-3 virus is a *Paramyxovirus*, also a member of the *Paramyxoviridae* family (Tsai *et al.*, Infect. Immun., 1975, **11**, 783-803).

Nucleotide sequences encoding the hemagglutinin and neuraminidase proteins (HN) and the fusion protein (F) of bPI-3 are known and accessible from the GenBank database under the accession number U31671.

The DNA vaccine or immunogenic or immunological composition against bPI-3 is preferably formulated with an adjuvant according to the invention, in particular DMRIE, preferably DMRIE-DOPE. This may be optionally combined with either the addition of bovine GM-CSF, or the optimization of at least one bPI-3 antigen, of finally the addition of bovine GM-CSF and the optimization of at least one bPI-3 antigen.

The addition of bovine GM-CSF may be carried out as is described for BHV-1.

The optimization of the antigens derived from bPI-3 is carried out by substitution, by a "signal" sequence, in particular that of the tPA of human origin, of the signal sequence of hemagglutinin-neuraminidase (HN) of bPI-3

and/or of the fusion protein (F) of bPI-3, and/or by the deletion of the DNA fragment encoding the transmembrane domain of HN and/or of F, and/or by the insertion of an intron, in particular of intron II of the rabbit beta-globin gene upstream of the nucleotide sequence encoding HN and/or F. The deletion of the DNA fragment encoding the transmembrane domain of one of these proteins is preferably accompanied by the contiguous C-terminal part. The DNA vaccine or immunogenic or immunological composition against bPI-3 according to the invention may therefore encode and express a single optimized PI-3 antigen (HN or F) or both (HN and F).

Nucleotide sequences encoding the bPI-3 antigens which can be used in the present invention and various expression vector constructs are given in the accompanying examples and in FR-A1-2751229, in particular in Examples 14 and 15, and in Figures 10 and 11.

Preferably, according to the invention, the DNA vaccine or immunogenic or immunological composition against bPI-3 is formulated with DMRIE-DOPE, and is composed of an expression plasmid (e.g. pLF1025 Example 7.1.2) encoding the HN antigen of bPI-3 optimized by the insertion of the signal sequence of the human tPA in place of the signal sequence of HN, by the deletion of the fragment of the nucleotide sequence of HN encoding the transmembrane domain and the contiguous C-terminal part and by the insertion of intron II of the rabbit beta-globin gene upstream of HN, and of a second expression plasmid (e.g. pLF1027 Example 7.2.2) encoding the F antigen of bPI-3 optimized by the insertion of the signal sequence of the human tPA in place of the signal sequence of F, by the deletion of the fragment of the nucleotide sequence encoding the

transmembrane domain of F and the contiguous C-terminal part and by the insertion of intron II of the rabbit beta-globin gene upstream of F.

The subject of the present invention is an improved DNA vaccine or immunogenic or immunological composition capable of inducing an improved or advantageously effective and/or protective immune response in pigs against porcine herpesvirus (PRV).

The PRV virus is a member of the *Alphaherpesvirinae* family, this virus is responsible for Aujeszky's disease (Sawitzky D., Arch. Virol. Suppl., 1997, **13**, 201-206).

Nucleotide sequences encoding the glycoproteins gB, gC and gD are known and accessible from the GenBank database under the accession number M17321, AF158090, AF086702.

The DNA vaccine or immunogenic or immunological composition against PRV is preferably formulated with an adjuvant according to the invention, in particular DMRIE, preferably DMRIE-DOPE. This may be optionally combined with either the addition of porcine GM-CSF (Inumaru S. and Takamatsu H., Immunol. Cell. Biol., 1995, **73**, 474-476), or the optimization of at least one PRV antigen, or finally the addition of porcine GM-CSF and the optimization of at least one PRV antigen.

The addition of porcine GM-CSF may be carried out by the incorporation of the porcine GM-CSF polypeptide into the vaccine or immunological or immunogenic composition or by the insertion of a nucleotide sequence encoding the porcine GM-CSF (e.g. accessible from the GenBank database under the accession number D21074) into an *in vivo* expression vector, preferably a plasmid. Preferably, the nucleotide sequence encoding porcine GM-CSF is inserted

into a second expression plasmid (e.g. pLF1033 Example 14), different from that (or those) into which the gene(s) encoding the PRV antigen(s) is (are) inserted.

The optimization of the antigens derived from PRV is carried out by substitution, by a "signal" sequence, in particular that of the tPA signal of human origin (GenBank accession number NM_000930), of the sequence of the signal peptide of the glycoprotein gB and/or of the glycoprotein gC and/or of the glycoprotein gD, and/or by the deletion of the DNA fragment encoding the transmembrane domain of gB and/or of gC and/or of gD. The deletion of the DNA fragment encoding the transmembrane domain of one of these glycoproteins is preferably accompanied by the contiguous C-terminal part. The DNA vaccine or immunological or immunogenic composition against PRV according to the invention may therefore encode and express a single optimized PRV antigen (gB, gC or gD) or two of them or the three, that is to say optimized gB, optimized gC and optimized gD.

Nucleotide sequences encoding the PRV antigens which can be used in the present invention and various expression vector constructs are given in the accompanying examples and in FR-A1-2751224, in particular in Examples 8 and 9 and in Figures 3 and 5.

Preferably, according to the invention, the DNA vaccine or immunogenic or immunological composition against PRV is formulated with DMRIE-DOPE, and is composed of an expression plasmid (e.g. pSB102 Example 8.1.2) encoding the gB antigen of PRV optimized by the deletion of the fragement of the nucleotide sequence encoding the transmembrane domain and of the contiguous C-terminal part, of a second expression plasmid (e.g. pSB104 Example 8.2.2)

encoding the gC antigen of PRV optimized by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain and of the contiguous C-terminal part, and of a third expression plasmid (e.g. pSB106 Example 8.3.2) encoding the gD antigen of PRV optimized by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain and of the contiguous C-terminal part.

The subject of the present invention is an improved DNA vaccine or immunogenic or immunological composition capable of inducing an improved or advantageously effective and/or protective immune response in pigs against the porcine reproductive respiratory syndrome virus (PRRSV).

The PRRSV virus is an Arterivirus, a member of the *Arteriviridae* family, (Murtaugh *et al.*, Arch. Virol., 1995, **140**, 1451-1460).

Nucleotide sequences encoding the proteins encoded by the open reading frames ORF3, ORF5 and ORF6 are known and accessible from the GenBank database under the accession number U87392.

The DNA vaccine or immunogenic or immunological composition against PRRSV is preferably formulated with an adjuvant according to the invention, in particular DMRIE, preferably DMRIE-DOPE. This may be optionally combined with either the addition of porcine GM-CSF, or the optimization of at least one PRRSV antigen, or finally the addition of porcine GM-CSF and the optimization of at least one PRRSV antigen.

The addition of porcine GM-CSF may be carried out as is described for PRV.

The optimization of the antigens derived from PRRSV is carried out by substitution, by a "signal" sequence, in

particular that of the tPA signal of human origin (GenBank accession number NM_000930), of the sequence of the signal peptide of the protein encoded by the open reading frame 3 (ORF3, gp45 or large envelope glycoprotein) and/or of the glycoprotein ORF5 (gp25 or envelope glycoprotein E) and/or of the glycoprotein ORF6 (gp18 or membrane protein), and/or by the deletion of the DNA fragment encoding the transmembrane domain of ORF3 and/or ORF5 and/or ORF6. The deletion of the DNA fragment encoding the transmembrane domain of one of these glycoproteins is preferably accompanied by the contiguous C-terminal part. The DNA vaccine or immunological or immunogenic composition against PRRSV according to the invention may therefore encode and express a single optimized PRRSV antigen (ORF3, ORF5 or ORF6) or two of them or the three, that is to say optimized ORF3, optimized ORF5 and optimized ORF6.

Nucleotide sequences encoding the PRRSV antigens which can be used in the present invention and various expression vector constructs are given in the accompanying examples and in FR-A1-2751224, in particular in Examples 14 to 17 and in Figures 14 to 17.

Preferably, according to the invention, the DNA vaccine or immunogenic or immunological composition against PRRSV is formulated with DMRIE-DOPE, and is composed of an expression plasmid (e.g. pLF1009 Example 9.1.1, pLF1015 Example 10.1.1) encoding the ORF3 antigen of PRRSV, of a second expression plasmid (e.g. pLF1012 Example 9.2.2, pLF1018 Example 10.2.2) encoding the ORF5 antigen of PRRSV optimized by substitution of the signal sequence of ORF5 by the human tPA signal peptide sequence and by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain and the contiguous C-terminal part,

and of a third expression plasmid (e.g. pLF1014 Example 9.3.2, pLF1016 Example 10.3.2) encoding the ORF6 antigen of PRRSV optimized by the substitution of the signal sequence of ORF6 by the human tPA signal peptide sequence and by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain and the contiguous C-terminal part.

A mixture of plasmids may be advantageously produced. The mixture may comprise at least two expression plasmids, each expressing a different immunogen (ORF3, ORF5 or ORF6) and/or obtained from a different strain of PRRSV (e.g. European strain, for example Lelystad, American strain ATCC VR-2332). In particular, a mixture made of six plasmids expressing PRRSV Lelystad ORF3, PRRSV Lelystad ORF5, PRRSV Lelystad ORF6, PRRSV VR-2332 ORF3, PRRSV VR-2332 ORF5 and PRRSV VR-2332 ORF6.

The subject of the present invention is also an improved DNA vaccine or immunogenic or immunological composition capable of inducing an improved or advantageously an effective and/or protective immune response in porcines against the swine influenza virus (SIV).

The SIV virus is an influenza virus group A, a member of the *Orthomyxoviridae* family (Murphy B.R. and Webster R.G., Virology, Second Edition, edited by B.N. Fields, D.M. Knipe *et al.*, Raven Press Ltd., New York 1990).

Nucleotide sequences encoding the hemagglutinin (HA) and neuraminidase (NA) proteins of the SIV H1N1 and H3N2 strains are known and accessible from the GenBank database under the accession number K00992, U86145, U07146, AF153238.

The DNA vaccine or immmunogenic or immunological composition against SIV is preferably formulated with an adjuvant according to the invention, in particular DMRIE, preferably DMRIE-DOPE. This may be optionally combined with either the addition of porcine GM-CSF, or the optimization of at least one SIV antigen, or finally the addition of porcine GM-CSF and the optimization of at least one SIV antigen.

The addition of porcine GM-CSF may be carried out as is described for PRV.

The optimization of the antigens derived from SIV is carried out by substitution, by a "signal" sequence, in particular that of the tPA of human origin, of the signal sequence of SIV hemagglutinin (HA) and/or of the SIV neuraminidase (NA) protein, and/or by the deletion of the DNA fragment encoding the transmembrane domain of HA and/or of NA, and/or by the insertion of an intron, in particular of intron II of the rabbit beta-globin gene upstream of the nucleotide sequence encoding HA and/or NA. The deletion of the DNA fragment encoding the transmembrane domain of one of these proteins is preferably accompanied by the contiguous C-terminal part. The DNA vaccine or immunological or immunogenic composition against SIV according to the invention may therefore encode and express a single optimized SIV antigen (HA or NA) or both (HA and NA).

Nucleotide sequences encoding SIV antigens which can be used in the present invention and various expression vector constructs are given in the accompanying examples and in FR-A1-2751224, in particular in Examples 10 and 11, and in Figures 7 and 9 for SIV strain H1N1, and in Examples 12 and 13, and in Figures 11 and 13 for SIV strain H3N2.

Preferably, according to the invention, the DNA vaccine or immunogenic or immunological composition against SIV is formulated with DMRIE-DOPE, and is composed of an expression plasmid (e.g. pLF1002 Example 11.1.2, pLF1006 Example 12.1.2) encoding the HA antigen of SIV optimized by the insertion of the signal sequence of the human tPA in place of the signal sequence of HA, by the deletion of the fragment of the nucleotide sequence of HA encoding the transmembrane domain and the contiguous C-terminal part, and by the insertion of intron II of the rabbit beta-globin gene upstream of HA, and of a second expression plasmid (e.g. pLF1004 Example 11.2.2, pLF1008 Example 12.2.2) encoding the NA antigen of SIV optimized by the insertion of the signal sequence of the human tPA in place of the signal sequence of NA, by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain of NA and the contiguous C-terminal part, and by the insertion of intron II of the rabbit beta-globin gene upstream of NA.

A mixture of plasmids may be advantageously produced. The mixture may comprise at least two expression plasmids, each expressing a different immunogen (HA or NA) and/or derived from a different SIV strain (e.g. H1N1 or H3N2). In particular, a mixture made of four plasmids expressing SIV H1N1 HA, SIV H1N1 NA, SIV H3N2 HA and SIV H3N2 NA.

Although the invention is described in relation to specific DNA vaccines or immunogenic or immunological compositions, the invention and in particular the use of the adjuvants according to the invention also applies to DNA vaccines or immunogenic or immunological compositions directed against other pathogens of these animal species.

In the same line of thought, the vaccines or compositions according to the invention may be, for an animal species, combined with one another and/or with DNA vaccines or immunogenic or immunological compositions directed against other pathogens of the same species.

These other pathogens may be in particular the rabies virus, hog cholera virus and porcine parvoviruses.

An immunogenic preparation or an improved DNA vaccine according to the invention against the rabies virus comprises in particular a plasmid encoding the unmodified G glycoprotein of the rabies virus and DMRIE-DOPE and optionally the addition of GM-CSF.

An improved immunogenic preparation or DNA vaccine according to the invention against the porcine parvovirus comprises in particular a plasmid encoding an antigen derived from the porcine parvovirus (e.g. the VP2 protein, Example 18 and Figure 18 of FR-A1-2751224) and DMRIE-DOPE and optionally the addition of porcine GM-CSF (e.g. pLF1033, Example 14).

An improved immunogenic preparation or DNA vaccine according to the invention against the hog cholera virus (HCV) comprises in particular a plasmid encoding an antigen derived from HCV (e.g. the E1 protein, Example 19 and Figure 19 of or the E2 protein, Example 20 and Figure 20 of the same document) and DMRIE-DOPE and optionally porcine GM-CSF (e.g. pLF1033, Example 14).

Thus, the subject of the present invention is also improved multivalent DNA vaccines or immunogenic or immunological compositions which make it possible to obtain an improved effect, advantageously, effective protection, in bovines against at least two bovine pathogens selected

from the group consisting of the BHV-1, BRSV, BVDV, bPI-3 and rabies viruses.

The subject of the present invention is also improved multivalent DNA vaccines or immunogenic or immunological compositions which make it possible to obtain an improved resonse and advantageously effective protection in pigs against at least two porcine pathogens selected from the group consisting of the PRV virus, PRRSV virus, SIV virus, hog cholera virus (or HCV), and porcine parvoviruses.

The multivalent DNA vaccines or immunogenic or immunological compositions may be improved by their formulation with an adjuvant according to the invention, in particular with DMRIE, preferably with DMRIE-DOPE. This may be optionally combined either with the addition of GM-CSF as previously described, or with the optimization of at least one antigen of interest as previously described, or finally by the addition of GM-CSF and the optimization of at least one antigen of interest.

The improved multivalent DNA vaccines or immunogenic or immunological compositions according to the invention are composed of one or more expression plasmids, such that these vaccines lead to the *in vivo* expression of at least one immunogen of a first pathogen and of at least one immunogen of at least one other pathogen, infecting the same animal species. At least one of these immunogens is preferably selected from the members of the following group:

- F of BRSV, G of BRSV, gB of BHV-1, gC of BHV-1, gD of BHV-1, E0 of BVDV-1, E2 of BVDV-1, E0 of BVDV-2, E2 of BVDV-2, F of bPI-3 and HN of bPI-3 for bovines, and

- gB of PRV, gC of PRV, gD of PRV, ORF3 of PRRSV strain Lelystad, ORF5 of PRRSV strain Lelystad, ORF6 of PRRSV strain Lelystad, ORF3 of PRRSSV strain VR-2332, ORF5 of PRRSV strain VR-2332, ORF6 of PRRSV strain VR-2332, HA of SIV strain H1N1, NA of SIV strain H1N1, HA of SIV strain H3N2 and NA of SIV strain H3N2 for porcines.

The improved monovalent or multivalent DNA vaccines or immunogenic or immunological compositions according to the invention may also be combined with at least one conventional vaccine or immunogenic or immunological composition (inactivated, attenuated live, subunit) and/or recombinant vaccine or immunogenic or immunological composotin using an *in vivo* expression vector (e.g. poxvirus, adenovirus, herpesvirus) directed against at least one pathogen - e.g., the same pathogen or a different pathogen - infecting the same species. Compositions can contain an improved vaccine or composition according to the invention and a conventional vaccine or immunogenic composition and/or a recombinant vaccine or immunogenic composition, wherein the conventional vaccine or immunogenic composition contains the same antigen or immunogen as the inventive improved vaccine or composition, e.g., if difected against the same pathogen, or a different antigen or immunogen, e.g., if directed against a different pathogen and/or the recombinant vaccine or composition expresses the same antigen or immunogen as the inventive improved vaccine or composition, e.g., if difected against the same pathogen, or a different antigen or immunogen, e.g., if directed against a different pathogen.

Analogously, the improved monovalent or multivalent DNA vaccines or immunogenic or immunological compositions according to the invention may also be administered

sequentially with at least one conventional vaccine (inactivated, attenuated live, subunit) and/or recombinant vaccine using an *in vivo* expression vector (e.g. poxvirus, adenovirus, herpesvirus) directed against at least one pathogen which can be the same pathogen or a different pathogen than that which is the subject of the improved vaccine or composition according to the invention; and, advantageously, the pathogens infect the same species. The improved vaccines or compositions of the invention can be used in "prime-boost" regimens; for instance, the improved vaccines or compositions of the invention can be administered first, with booster or subsequent administration(s) of inventive vaccine(s) or composition(s) and/or conventional vaccine(s) and/or recombinant vaccine(s); or, the conventional and/or recombinant vaccine(s) or composition(s) can be initially administered with booster or subsequent administration(s) of inventive vaccine(s) or composition(s). The immunogen or antigen contained in or expressed by the conventional or recombinant vaccine or composition can be the same as that expressed by the herein inventive improved vaccine or composition; or , the immunogen or antigen contained in or expressed by the conventional or recombinant vaccine or composition can be from the same pathogen as that expressed by the herein inventive improved vaccine or composition; or the immunogen or antigen contained in or expressed by the conventional or recombinant vaccine or composition can be from a different pathogen as that expressed by the herein inventive improved vaccine or composition, but advantageously the pathogen effects the same species.

Accordingly, the invention also envisions kits containing the improved monovalent or multivalent DNA

vaccines or immunogenic or immunological compositions according to the invention and at least one conventional vaccine (inactivated, attenuated live, subunit) and/or recombinant vaccine using an *in vivo* expression vector; for instance, for combination or sequential administration. The vaccine(s) or composition(s) are advantageously in separate containers. The separate containers can be packaged together. The kit can contain instructions for admixture and/or administration; for instance, instructions for admixture and administration of the mixture or for sequential or prime-boost administration.

Persons skilled in the art may refer to FR-A1-2751229 for the methods for constructing the plasmids containing these bovine valencies, to FR-A1-2751224 for the porcine valencies.

The subject of the present invention is also a method of vaccinating farm animals, in particular bovines or porcines. This vaccination method comprises the administration of one of the monovalent or multivalent improved DNA vaccines as described above. These vaccination methods concern gestating females for the passive transfer of immunity or young animals or adults. This vaccination method comprises the administration of one or more doses of the improved DNA vaccine.

The quantity of DNA used in the vaccines according to the present invention is between about 10 µg and about 1000 µg, and preferably between about 50 µg and about 500 µg, for a given plasmid. Persons skilled in the art possess the competence necessary to precisely define the effective dose of DNA to be used for each vaccination protocol.

The dose volumes may be preferably between 0.2 and 5 ml, preferably between 1 and 3 ml.

The improved DNA vaccines according to the invention may be administered, in the context of this vaccination method, by various routes of administration proposed in the prior art for polynucleotide vaccination and by means of known techniques of administration.

According to a preferred mode of the invention, the methods of vaccination comprise the administration of the improved DNA vaccines according to the invention by the intramuscular route, the subcutaneous route or with the aid of an injector without needle by the intradermal route.

The invention will now be described in greater detail with the aid of embodiments taken as nonlimiting examples and referring to the drawings, in which:

Figure No. 1: plasmid pVR1012

Figure No. 2: plasmid pAB110

Sequence listing:

SEQ ID NO 1: oligonucleotide PB326

SEQ ID NO 2: oligonucleotide PB329

SEQ ID NO 3: oligonucleotide SB090

SEQ ID NO 4: oligonucleotide SB091

SEQ ID NO 5: oligonucleotide LF001

SEQ ID NO 6: oligonucleotide LF002

SEQ ID NO 7: oligonucleotide PB234

SEQ ID NO 8: oligonucleotide PB235

SEQ ID NO 9: oligonucleotide PB511

SEQ ID NO 10: oligonucleotide PB512

SEQ ID NO 11: oligonucleotide SB221

SEQ ID NO 12: oligonucleotide SB222

SEQ ID NO 13: oligonucleotide PB507

SEQ ID NO 14: oligonucleotide PB508
SEQ ID NO 15: oligonucleotide PB513
SEQ ID NO 16: oligonucleotide PB514
SEQ ID NO 17: oligonucleotide SB223
SEQ ID NO 18: oligonucleotide SB224
SEQ ID NO 19: oligonucleotide PB497
SEQ ID NO 20: oligonucleotide PB498
SEQ ID NO 21: oligonucleotide SB225
SEQ ID NO 22: oligonucleotide SB226
SEQ ID NO 23: oligonucleotide SB210
SEQ ID NO 24: oligonucleotide SB211
SEQ ID NO 25: oligonucleotide SB212
SEQ ID NO 26: oligonucleotide SB220
SEQ ID NO 27: oligonucleotide SB213
SEQ ID NO 28: oligonucleotide SB214
SEQ ID NO 29: oligonucleotide SB215
SEQ ID NO 30: oligonucleotide SB216
SEQ ID NO 31: oligonucleotide LF050
SEQ ID NO 32: oligonucleotide LF051
SEQ ID NO 33: oligonucleotide LF052
SEQ ID NO 34: oligonucleotide LF053
SEQ ID NO 35: oligonucleotide LF039
SEQ ID NO 36: oligonucleotide LF040
SEQ ID NO 37: oligonucleotide LF041
SEQ ID NO 38: oligonucleotide LF042
SEQ ID NO 39: oligonucleotide LF043
SEQ ID NO 40: oligonucleotide LF044
SEQ ID NO 41: oligonucleotide LF045
SEQ ID NO 42: oligonucleotide LF046
SEQ ID NO 43: oligonucleotide LF064
SEQ ID NO 44: oligonucleotide LF065
SEQ ID NO 45: oligonucleotide LF066

SEQ ID NO 46: oligonucleotide LF067
SEQ ID NO 47: oligonucleotide LF047
SEQ ID NO 48: oligonucleotide LF048
SEQ ID NO 49: oligonucleotide LF058
SEQ ID NO 50: oligonucleotide LF059
SEQ ID NO 51: oligonucleotide LF060
SEQ ID NO 52: oligonucleotide LF061
SEQ ID NO 53: oligonucleotide LF062
SEQ ID NO 54: oligonucleotide LF063
SEQ ID NO 55: oligonucleotide SB201
SEQ ID NO 56: oligonucleotide SB202
SEQ ID NO 57: oligonucleotide SB203
SEQ ID NO 58: oligonucleotide SB217
SEQ ID NO 59: oligonucleotide SB204
SEQ ID NO 60: oligonucleotide SB205
SEQ ID NO 61: oligonucleotide SB206
SEQ ID NO 62: oligonucleotide SB218
SEQ ID NO 63: oligonucleotide SB207
SEQ ID NO 64: oligonucleotide SB208
SEQ ID NO 65: oligonucleotide SB209
SEQ ID NO 66: oligonucleotide SB219
SEQ ID NO 67: oligonucleotide LF027
SEQ ID NO 68: oligonucleotide LF028
SEQ ID NO 69: oligonucleotide LF019
SEQ ID NO 70: oligonucleotide LF020
SEQ ID NO 71: oligonucleotide LF021
SEQ ID NO 72: oligonucleotide LF022
SEQ ID NO 73: oligonucleotide LF023
SEQ ID NO 74: oligonucleotide LF024
SEQ ID NO 75: oligonucleotide LF025
SEQ ID NO 76: oligonucleotide LF026
SEQ ID NO 77: oligonucleotide LF037

SEQ ID NO 78: oligonucleotide LF038

SEQ ID NO 79: oligonucleotide LF029

SEQ ID NO 80: oligonucleotide LF030

SEQ ID NO 81: oligonucleotide LF031

SEQ ID NO 82: oligonucleotide LF032

SEQ ID NO 83: oligonucleotide LF033

SEQ ID NO 84: oligonucleotide LF034

SEQ ID NO 85: oligonucleotide LF035

SEQ ID NO 86: oligonucleotide LF036

SEQ ID NO 87: oligonucleotide LF003

SEQ ID NO 88: oligonucleotide LF004

SEQ ID NO 89: oligonucleotide LF005

SEQ ID NO 90: oligonucleotide LF006

SEQ ID NO 91: oligonucleotide LF007

SEQ ID NO 92: oligonucleotide LF008

SEQ ID NO 93: oligonucleotide LF009

SEQ ID NO 94: oligonucleotide LF010

SEQ ID NO 95: oligonucleotide LF011

SEQ ID NO 96: oligonucleotide LF012

SEQ ID NO 97: oligonucleotide LF013

SEQ ID NO 98: oligonucleotide LF014

SEQ ID NO 99: oligonucleotide LF015

SEQ ID NO 100: oligonucleotide LF016

SEQ ID NO 101: oligonucleotide LF017

SEQ ID NO 102: oligonucleotide LF018

SEQ ID NO 103: oligonucleotide LF054

SEQ ID NO 104: oligonucleotide LF055

SEQ ID NO 105: oligonucleotide LF056

SEQ ID NO 106: oligonucleotide LF057

EXAMPLES:

For each of the pathogens considered, each gene encoding the principal antigens (native form and modified form) was the subject of a particular construction in a eukaryotic expression plasmid. The secreted forms of the antigens were obtained by deletion of the fragments of genes encoding the transmembrane and cytoplasmic domains. In all cases, the transmembrane domains of the proteins were identified on the basis of the hydropathy profiles (on MacVector 6.5) of the corresponding protein sequences.

Example 1: Molecular biology methods

1.1 Extraction of viral genomic DNA

Viral suspensions were treated with proteinase K (100 mg/ml final) in the presence of sodium dodecyl sulphate (SDS) (0.5% final) for 2 hours at 37°C. The viral DNA was then extracted with the aid of a phenol/chloroform mixture, and then precipitated with two volumes of absolute ethanol at -20°C for 16 hours and then centrifuged at 10,000 g for 15 minutes at 4°C. The DNA pellets were dried, and then taken up in a minimum volume of sterile ultrapure water.

1.2 Isolation of viral genomic RNA

The genomic RNA of each virus was extracted using the "guanidinium thiocyanate/phenol-chloroform" technique described by P. Chomczynski and N. Sacchi (Anal. Biochem. 1987. **162**. 156-159).

1.3 Molecular biology techniques

All the constructions of plasmids were carried out using the standard molecular biology techniques described

by Sambrook *et al.* (Molecular Cloning: A Laboratory Manual. 2nd Edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989). All the restriction fragments used for the present invention were isolated with the aid of the "Geneclean" kit (BIO101 Inc., La Jolla, CA). For all the constructs, the cloned DNA fragments, as well as the junctions with the expression vector, were sequenced by the Sanger method (Sambrook *et al.*, 1989).

1.4 PCR and RT-PCR

The oligonucleotides specific to the genes or gene fragments cloned were synthesized, some of them containing, in some cases, at their 5' end, restriction sites facilitating the cloning of the amplified fragments. The reverse transcription (RT) reactions and the polymerase chain reaction (PCR) were carried out according to standard techniques (Sambrook *et al.*, 1989).

1.5 Large-scale purification of plasmids

The production, on the scale of about ten mg, of purified plasmids entering into the vaccinal compositions was carried out by the caesium chloride-ethidium bromide gradient method (Sambrook *et al.*, 1989).

Example 2: Basic plasmid constructs

The eukaryotic expression plasmid pVR1020 (C.J. Luke *et al.* J. of Infectious Diseases, 1997, **175**, 95-97), derived from the plasmid pVR1012 (Figure No. 1, Figure 1 and Example 7 of WO-A-9803199), contains the coding phase of the signal sequence of the human tissue plasminogen activator (tPA).

A plasmid pVR1020 is modified by BamHI-BglII digestion and insertion of a sequence containing several cloning sites (BamHI, NotI, EcoRI, XbaI, PmII, PstI, BglII) and resulting from the pairing of the following oligonucleotides:

PB326 (40 mer) (SEQ ID NO 1)

5' GATCTGCAGCACGTGTCTAGAGGATATCGAATTCGCGGCC 3' and

PB329 (40 mer) (SEQ ID NO 2)

5' GATCCGCGGCCGCGAATTCGATATCCTCTAGACACGTGCT 3'.

The vector thus obtained, having a size of about 5105 base pairs (or bp), is called pAB110 (Figure No. 2).

Intron II of the rabbit β-globin gene is cloned into the vector pCRII (Invitrogen, Carlsbad, CA, USA) after production of the corresponding DNA fragment by PCR with the aid of the following oligonucleotides:

SB090 (20 mer) (SEQ ID NO 3)

5' TTGGGGACCCTTGATTGTTC 3' and

SB091 (21 mer) (SEQ ID NO 4)

5' CTGTAGGAAAAAGAAGAAGGC 3'

using as template the genomic DNA of rabbit peripheral blood cells. The resulting plasmid is designated pNS050.

The expression plasmid pAB110 is modified by introducing the sequence of intron II of the rabbit globin gene into the SalI site situated upstream of the ATG of the signal peptide of tissue plasminogen activator (tPA). The sequence of intron II of the rabbit globin gene is amplified by polymerase chain reaction (PCR) from the plasmid pNS050 using the following oligonucleotide pair:

LF001 (30 mer) (SEQ ID NO 5)

5' CTCCATGTCGACTTGGGGACCCTTGATTGT 3' and

LF002 (30 mer) (SEQ ID NO 6)

5' CTCCATGTCGACCTGTAGGAAAAAGAAGAA 3'

The PCR product (573 base pairs or bp) is digested with SalI and cloned into the plasmid pAB110 previously linearized with SalI, to generate the plasmid pLF999 of about 5678 bp.

Example 3: Plasmids encoding the various forms of the bovine herpesvirus type 1 (BHV-1) antigens

Fragments of viral DNA containing the gB, gC and gD genes of the B901 strain of BHV-1 are isolated by digesting the viral genome with various restriction enzymes, by separating them by agarose gel electrophoresis and by analysing them by Southern blotting with the aid of probes corresponding to fragments of the gB, gC and gD genes of the ST strain of BHV-1 (Leung-Tack P. *et al.*, Virology, 1994, **199**, 409-421). The BHV-1 Colorado strain [Cooper] (ATCC number VR-864) can also be used. The fragments thus identified are cloned into the vector pBluescript SK+ (Stratagene, La Jolla, CA, USA) and are at the origin of the clonings of the three genes into the expression vector pVR1012.

3.1 Plasmids encoding the various forms of BHV-1 gB

3.1.1 pPB280: gB gene (native form) cloned into the vector pVR1012

Two XhoI-XhoI fragments containing the 5' and 3' portions of the BHV-1 gB gene are identified by Southern blotting and cloned into the vector pBluescript SK+ (Stratagene, La Jolla, CA, USA) previously digested with XhoI. The plasmids thus obtained are designated pPB128 and pPB117 respectively.

The plasmid pPB128, containing the 5' fragment of the gB gene, is digested with NotI and XhoI, generating a fragment of 1708 bp (fragment A).

The plasmid pPB117, containing the 3' portion of the gB gene, is digested with XhoI and StuI, generating a fragment of 1345 bp. The latter fragment is cloned into the vector pBluescript KS+ (Stratagene, La Jolla, CA, USA) previously digested with EcoRV and XhoI. The resulting plasmid is called pPB279. The plasmid pPB279 is then digested with XhoI and BamHI, generating a DNA fragment of 1413 bp (fragment B).

Fragments A and B are then cloned into a vector pBluescript KS+ digested with NotI and BamHI, generating plasmid pPB278 (about 6063 bp) and allowing the reconstitution of the BHV-1 gB gene.

The vector pPB278 then serves as template during a PCR reaction carried out with the following oligonucleotides:

PB234 (30 mer) (SEQ ID NO 7)

5' TTGTCGACATGGCCGCTCGCGGCGGTGCTG 3' and

PB235 (21 mer) (SEQ ID NO 8)

5' GCAGGGCAGCGGCTAGCGCGG 3'.

The PCR product (146 bp) is then digested with the restriction enzymes SalI and NheI.

The plasmid pPB278 is digested with NheI and BamHI. The fragment of 2728 bp thus obtained and the PCR fragment previously digested are ligated into the vector pVR1012 (Example 2) previously digested with SalI and BamHI, thus generating the plasmid pPB280, having a size of about 7742 bp.

The BHV-1 gB gene encodes a protein of 933 amino acids.

3.1.2 pPB281: gB gene (Δ[TM-Cter] form) cloned into the vector pVR1012

The truncated form (deleted for its transmembrane (TM) and carboxy-terminal (Cter) domains) of the BHV-1 gB gene is obtained by ligating into the plasmid pVR1012 (Example 2) predigested with SalI and BamHI, both a fragment having a size of 2234 bp obtained after digestion with SalI-PvuII of the plasmid pPB280 (Example 3.1.1) and a fragment of 56 bp obtained by pairing of the following oligonucleotides:

PB511 (52 mer) (SEQ ID NO 9)

5' CTGCACGAGCTCCGGTTCTACGACATTGACCGCGTGGTCAAGACGGACTGAG 3'

and

PB512 (57 mer) (SEQ ID NO 10)

5' GATCCTCAGTCCGTCTTGACCACGCGGTCAATGTCGTAGAACCGGAGCTCGTGCAG 3'.

The plasmid thus generated has a size of about 7154 bp and is called pPB281. The truncated gB gene of BHV-1 encodes a protein of 759 amino acids.

3.1.3 pSB115: gB gene (tPA Δ[TM-Cter] form) cloned into the vector pAB110

The tPA Δ[TM-Cter] form of the BHV-1 gB gene is amplified by PCR from the template pPB281 (Example 3.1.2) and with the aid of the following primers:

SB221 (39 mer) (SEQ ID NO 11)

5' AAAATTTCGATATCCGCCGCGGGGCGACCGGCGACAACG 3' and

SB222 (33 mer) (SEQ ID NO 12)

5' GGAAGATCTTCAGTCCGTCTTGACCACGCGGTC 3'

The amplification product (2088 bp) is digested with the enzymes EcoRV and BglII and cloned into the vector

pAB110 (Example 2) previously digested with EcoRV and BglII, generating the plasmid pSB115, having a size of about 7154 bp.

The tPA Δ[TM-Cter] form of the gB gene encodes a glycoprotein of 729 amino acids, containing the extracellular domain of the BHV-1 gB glycoprotein.

3.2. Plasmids encoding the various forms of BHV-1 gC

3.2.1 pPB264: gC gene (native form) cloned into the vector pVR1012

A BamHI-HindIII fragment of 3.5 kb containing the complete BHV-1 gC gene is identifed by Southern blotting and cloned into the vector pBluescript SK+. The plasmid thus obtained is called pPB287.

The plasmid pPB287 is then digested with NcoI-BssSI. A digestion fragment having a size of 1492 bp is obtained. It is ligated with a synthetic DNA fragment obtained by the pairing of the following oligonucelotides:

PB507 (37 mer) (SEQ ID NO 13)
5' TCGTGCCTGCGGCGCAAGGCCCGGGCGCGCCTGTAGT 3' and
PB508 (37 mer) (SEQ ID NO 14)
5' CTAGACTACAGGCGCGCCCGGGCCTTGCGCCGCAGGC 3',

into the plasmid pLitmus 28 (New England Biolabs, Inc., Beverly, MA, USA) predigested with NcoI and XbaI, generating the intermediate plasmid pPB290.

The fragment of 1554 bp derived from the digestion of pPB290 with PstI and XbaI is cloned into the vector pVR1012 (Example 2) previously digested with PstI and XbaI, thus generating the plasmid pPB264, having a size of about 6427 bp. The BHV-1 gC gene encodes a protein of 508 amino acids.

3.2.2 pPB292: gC gene (Δ[TM-Cter] form) cloned into the vector pVR1012

The truncated form of the BHV-1 gC gene is obtained by ligating the following three DNA fragments into the vector pVR1012 (Example 2) previously digested with PstI and XbaI:

(a) a fragment of 1035 bp derived from the digestion of pPB264 (Example 3.2.1) with PstI and XhoI,

(b) a fragment of 350 bp derived from the digestion of pPB264 with XhoI and BanI and

(c) a synthetic fragment of 43 bp resulting from the pairing of the oligonucleotides PB513 and PB514.

These oligonucleotides are the following:

PB513 (43 mer) (SEQ ID NO 15)

5' GCACCGCTGCCCGAGTTCTCCGCGACCGCCACGTACGACTAGT 3' and

PB514 (43 mer) (SEQ ID NO 16)

5' CTAGACTAGTCGTACGTGGCGGTCGCGGAGAACTCGGGCAGCG 3'.

The plasmid having a size of about 6305 bp thus obtained is called pPB292. The truncated gC gene of BHV-1 encodes a protein of 466 amino acids.

3.2.3 pSB116: gC gene (tPA Δ[TM-Cter] form) cloned into the vector pAB110

The tPA Δ[TM-Cter] form of the BHV-1 gC gene is amplified by PCR from the template pPB292 (Example 3.2.2) and with the aid of the following primers:

SB223 (39 mer) (SEQ ID NO 17)

5' AAAATTTCGATATCCCGGCGGGGGCTCGCCGAGGAGGCG 3' and

SB224 (32 mer) (SEQ ID NO 18)

5' GGAAGATCTCTAGTCGTACGTGGCGGTCGCGG 3'

The amplification product (1362 bp) is digested with the enzymes EcoRV and BglII and cloned into the vector pAB110 (Example 2) previously digested with EcoRV and BglII, generating the plasmid pSB116, having a size of about 6404 bp.

The tPA Δ[TM-Cter] form of the gC gene encodes a glycoprotein of 479 amino acids, containing the extracellular domain of the BHV-1 gC glycoprotein.

3.3 Plasmids encoding the various forms of BHV-1 gD

3.3.1 pPB148: gD gene (native form) cloned into the vector pVR1012

A XhoI-XhoI fragment of 5 kb containing the BHV-1 gD gene is identified by Southern blotting and cloned into the vector pBluescript SK+ predigested with XhoI, generating the plasmid pPB147.

A fragment of 325 bp derived from the digestion of pPB147 with NdeI and BsrBI and a fragment of 943 bp derived from the digestion of pPB147 with NdeI and StyI are then ligated into the vector pVR1012 (Example 2) predigested with EcoRV and XbaI, thus generating the plasmid pPB148, having a size of about 6171 bp. The BHV-1 gD gene encodes a protein of 417 amino acids.

3.3.2 pPB284: gD gene (Δ[TM-Cter] form) cloned into the vector pVR1012

The truncated gD gene of BHV-1 is obtained from a fragment obtained after PCR amplification carried out on the genomic DNA of the B901 strain of the BHV-1 virus previously digested with PstI and XbaI and with the aid of the following primer pair:

PB497 (33 mer) (SEQ ID NO 19)
5' TTTCTGCAGATGCAAGGGCCGACATTGGCCGTG 3' and
PB498 (31 mer) (SEQ ID NO 20)
5' TTTCTAGATTAGGGCGTAGCGGGGGCGGGCG 3'.

This PCR fragment is then cloned into the plasmid pVR1012 (Example 2) previously digested with PstI and XbaI, generating the plasmid pPB284, having a size of about 5943 bp. The truncated gD gene of BHV-1 encodes a protein of 355 amino acids.

3.3.3 pSB117: gD gene (tPA Δ[TM-Cter] form) cloned into the vector pAB110

The tPA Δ[TM-Cter] form of the BHV-1 gD gene is amplified by PCR from the pPB284 template (Example 3.3.2) and with the aid of the following primers:
SB225 (39 mer) (SEQ ID NO 21)
5' AAAATTTCGATATCCCCCGCGCCGCGGGTGACGGTATAC 3' and
SB226 (33 mer) (SEQ ID NO 22)
5' GGAAGATCTTTAGGGCGTAGCGGGGGCGGGCGG 3'.

The amplification product (1029 bp) is digested with the enzymes EcoRV and BglII and cloned into the vector pAB110 (Example 2) previously digested with EcoRV and BglII, generating the plasmid pSB117, having a size of about 6071 bp.

The tPA Δ[TM-Cter] form of the gD gene encodes a glycoprotein of 368 amino acids, containing the extracellular domain of the BHV-1 gD glycoprotein.

Example 4: Plasmids encoding the various forms of the bovine respiratory sencitial virus (BRSV) antigens

The genes encoding the F and G antigens of the BRSV virus are obtained by RT-PCR from the viral RNA of the

Snook strain (Thomas *et al.* Research in Vet. Science, 1982, **33**, 170-182). The BRSV A 51908 strain (ATCC number VR-794) may also be used.

4.1 Plasmids encoding the various forms of BRSV-F

4.1.1 pSB107: F gene (native form) cloned into the vector pVR1012

The F gene of the Snook strain of BRSV is amplified by RT-PCR using the viral RNA as template and with the aid of the following primers:

SB210 (34 mer) (SEQ ID NO 23)
5' AAATTTTCTGCAGATGGCGACAACAGCCATGAGG 3' and
SB211 (35 mer) (SEQ ID NO 24)
5' TTAAGGATCCTCATTTACTAAAGGAAAGATTGTTG 3'.

The amplification product, having a size of 1739 bp, is digested with the enzymes PstI and BamHI and cloned into the vector pVR1012 (Example 2) previously digested with PstI and BamHI, thus generating the plasmid pSB107, having a size of about 6583 bp.

The F gene of the BRSV virus encodes a protein of 574 amino acids.

4.1.2 pSB108: F gene (Δ[TM-Cter] form) cloned into the vector pVR1012

The truncated form of the F gene of the Snook strain of BRSV is amplified by RT-PCR using the viral RNA as template and with the aid of the following primers:

SB210 (SEQ ID NO 23) and
SB212 (39 mer) (SEQ ID NO 25)
5' AATTTTGGATCCTCATGTGGTGGATTTTCCTACATCTAC 3'.

The amplification product (1581 bp) is digested with the enzymes PstI and BamHI and cloned into the vector pVR1012 (Example 2) previously digested with PstI and BamHI, generating the plasmid pSB108, having a size of about 6430 bp.

The truncated form of the F gene encodes a glycoprotein of 523 amino acids, containing the extracellular domain of the BRSV F glycoprotein.

4.1.3 pSB114: F gene (tPA Δ[TM-Cter] form) cloned into the vector pAB110

The tPA Δ[TM-Cter] form of the F gene of the BRSV Snook strain is amplified by RT-PCR using the viral RNA as template and with the aid of the following primers:

SB212 (SEQ ID NO 25) and

SB220 (38 mer) (SEQ ID NO 26)

5' AAAATTCACGTGAACATAACAGAAGAATTTTATCAATC 3'.

The amplification product (1516 bp) is digested with the enzymes PmlI and BglII and cloned into the vector pAB110 (Example 2) previously digested with PmlI and BglII, generating the plasmid pSB114, having a size of about 6572 bp.

The tPA Δ[TM-Cter] form of the F gene encodes a glycoprotein of 535 amino acids, containing the extracellular domain of the BRSV F glycoprotein.

4.2 Plasmids encoding the various forms of BRSV-G

In the case of the BRSV G protein (type II glycoprotein), the signal sequence and the transmembrane sequence are indistinguishable, requiring the addition of a signal sequence upstream of the sequence corresponding to

the extracellular domain during the deletion of the transmembrane domain.

The plasmid pAB110 (Example 2) is used for the construction of the plasmids containing the truncated forms of the gene encoding the BRSV G protein.

4.2.1 pSB109: G gene (native form) cloned into the vector pVR1012

The G gene of the BRSV Snook strain is amplified by RT-PCR using the viral RNA as template and with the aid of the following primers:

SB213 (32 mer) (SEQ ID NO 27)
5' ACGCGTCGACATGTCCAACCATACCCATCATC 3' and
SB214 (38 mer) (SEQ ID NO 28)
5' TTAAAATCTAGATTAGATCTGTGTAGTTGATTGATTTG 3'.

The amplification product (784 bp) is digested with enzymes SalI and XbaI and cloned into the vector pVR1012 (Example 2) previously digested with SalI and XbaI, generating the plasmid pSB109, having a size of about 5661 bp.

The BRSV G gene encodes a glycoprotein of 257 amino acids.

4.2.2 pSB110: G gene (tPA Δ[TM-Cter] form) cloned into the vector pAB110

The truncated form of the G gene of the BRSV Snook strain is amplified by RT-PCR using the viral RNA as template and with the aid of the following primers:

SB215 (33 mer) (SEQ ID NO 29)
5' TTTTAAGGATCCGCTAAAGCCAAGCCCACATCC 3' and
SB216 (33 mer) (SEQ ID NO 30)
5' TTAAAATCTAGATTAGATCTGTGTAGTTGATTG 3'.

The amplification product (666 bp) is digested with the enzymes BamHI and XbaI and cloned into the vector pAB110 (Example 2) previously digested with BamHI and XbaI, generating the plasmid pSB110, having a size of about 5660 bp.

The tPA Δ[TM-Cter] form of the BRSV virus G gene encodes a glycoprotein of 218 amino acids, containing the extracellular domain of the G glycoprotein, but preceded by the signal sequence of the tissue plasminogen activator.

Example 5: Plasmids encoding the various forms of the bovine viral diarrhea virus type 1 (BVD-1) antigens

The genes encoding the E0 (glycoprotein of 48 kDa or gp48) and E2 (gp53) antigens of the type 1 BVDV viruses are obtained by RT-PCR from the viral RNA of the Osloss strain (L. De Moerlooze *et al.* J. Gen. Virol. 1993, **74**, 1433-1438; A. Renard *et al.*, DNA, 1985, **4**, 439-438; A. Renard *et al.* Ann. Rech. Vet., 1987, **18**, 121-125). The NADL (ATCC VR-534) or New York (ATCC VR-524) strains may also be used.

5.1 Plasmids encoding the various forms of E0 of the BVDV type 1 Osloss strain

5.1.1 pLF1028: E0 gene (native form) cloned into the vector pVR1012

The complementary DNA (cDNA) of the E0 gene of the Osloss strain is synthesized from the corresponding viral RNA with the aid of the primer LF051 and amplified by the PCR reaction with the aid of the following oligonucleotide pair:

LF050 (36 mer) (SEQ ID NO 31)

5' CATACCGTCGACATGAAGAAACTAGAGAAAGCCCTG 3' and
LF051 (40 mer) (SEQ ID NO 32)
5' CATACCGGATCCTCAGGCTGCATATGCCCCAAACCATGTC 3'.

The DNA fragment of about 765 bp obtained by digesting the PCR product with SalI and BamHI is ligated with a fragment of 4866 bp resulting from the digestion of pVR1012 (Example 2) with SalI and BamHI in order to generate the plasmid pLF1028 (about 5636 bp). The E0 gene of BVDV-1 strain Osloss encodes a protein of 252 amino acids.

An ATG codon is introduced into the sequence of the oligonucleotide LF050 so as to allow the initiation of the translation of the corresponding recombinant E0 polypeptide.

5.1.2 pLF1029: E0 gene, (β-globin tPA-E0) form cloned into the vector pLF999.

The E0 gene is synthesized by a PCR reaction from the pLF1028 template (Example 5.1.1) and with the aid of the following oligonucleotide pair:
LF052 (39 mer) (SEQ ID NO 33)
5' CATGACGCGGCCGCTATGAAGAAACTAGAGAAAGCCCTG 3' and
LF053 (40 mer) (SEQ ID NO 34)
5' CATGACAGATCTTTAGGCTGCATATGCCCCAAACCATGTC 3'.

The DNA fragment of about 770 bp obtained by digesting the PCR product with NotI and BglII is ligated with a fragment of 5642 bp resulting from the digestion of pLF999 (Example 2) with NotI and BglII in order to generate the plasmid pLF1029 (about 6417 bp).

The E0 gene of BVDV-1 strain Osloss thus modified (β-globin tPA-E0) encodes a protein of 283 amino acids.

5.2 Plasmids encoding the various forms of E2 of the BVDV type 1 Osloss strain

5.2.1 pLF1020: E2 gene (native form) cloned into the vector pVR1012

The cDNA of the E2 gene of the Osloss strain is synthesized from the corresponding viral RNA with the aid of the primer LF040 and amplified by a PCR reaction with the aid of the following oligonucleotide pair:

LF039 (33 mer) (SEQ ID NO 35)

5' CATGACGTCGACATGACGACTACTGCATTCCTG 3' and

LF040 (36 mer) (SEQ ID NO 36)

5' CATGACAGATCTTCAACGTCCCGAGGTCATTTGTTC 3'.

The DNA fragment of 1235 bp obtained by digesting the PCR product with SalI and BglII is ligated with a fragment of 4860 bp resulting from the digestion of pVR1012 (Example 2) with SalI and BglII in order to generate the plasmid pLF1020 (about 6100 pb).

The E2 gene of BVDV-1 strain Osloss encodes a protein of 409 amino acids.

An ATG codon is introduced into the sequence of the oligonucleotide LF039 so as to allow the initiation of the translation of the corresponding recombinant E2 polypeptide.

5.2.2 pLF1021: E2 gene, (β-globin tPA-E2 Δ[TM+Cter]) form cloned into the vector pLF999.

The E2 gene deleted for its transmembrane and carboxy-terminal domains is synthesized by a PCR reaction from the pLF1020 template (Example 5.2.1) and with the aid of the following oligonucleotide pair:

LF041 (36 mer) (SEQ ID NO 37)

5' CATGACGCGGCCGCTATGACGACTACTGCATTCCTG 3' and

LF042 (35 mer) (SEQ ID NO 38)

5' CATGACAGATCTCAAGCGAAGTAATCCCGGTGGTG 3.

The DNA fragment of 1132 bp obtained by digesting the PCR product with NotI and BglII is ligated with a fragment of 5642 bp resulting from the digestion of pLF999 (Example 2) with NotI and BglII in order to generate the plasmid pLF1021 (about 6779 bp).

The E2 gene of BVDV-1 strain Osloss thus modified (β-globin tPA-E2 Δ[TM+Cter]) encodes a protein of 404 amino acids.

Example 6: Plasmids encoding the various forms of the bovine viral diarrhea virus type 2 (BVDV-2) antigens

The genes encoding the E2 antigen (gp53) of the BVDV type 2 viruses are obtained by RT-PCR from the viral RNA of the strain 890 (J.F. Ridpath and S.R. Bolin, Virology, 1995, **212**, 36-46). The strain Q140 can also be used and may be obtained from the Quebec Ministry of Agriculture, Fisheries and Food, Armand-Frappier Institute (P. Tijssen *et al.*, Virology, 1996, **217**, 356-361). The strains 1373 and 296 may also be used (J.F. Ridpath, BVDV Research Project, National Animal Disease Center, 2300 Dayton Avenue, Ames, USA).

6.1 Plasmids encoding the various forms of E2 of the type 2 - 890 strain

6.1.1. pLF1022: E2 gene (native form) cloned into the vector pVR1012

The cDNA of the E2 gene of the strain 890 is synthesized from the corresponding viral RNA with the aid

of the primer LF044 amplified by a PCR reaction with the aid of the following oligonucleotide pair:

LF043 (36 mer) (SEQ ID NO 39)

5' ACTGTATCTAGAATGACCACCACAGCTTTCCTAATC 3' and

LF044 (39 mer) (SEQ ID NO 40)

5' ACTGTAAGATCTTTAAGTATTCACTCCAGCACCCATAGC 3'.

The DNA fragment of about 1240 bp obtained by digesting the PCR product with XbaI and BglII is ligated with a fragment of 4891 bp resulting from the digestion of pVR1012 (Example 2) with XbaI and BglII in order to generate the plasmid pLF1022 (about 6136 bp).

The E2 gene of BVDV-2 strain 890 encodes a protein of 410 amino acids.

An ATG codon is introduced into the sequence of the oligonucleotide LF043 so as to allow the initiation of the translation of the corresponding recombinant E2 polypeptide.

6.1.2 pLF1023: E2 gene, (β-globin tPA-E2 Δ[TM+Cter]) form, cloned into the vector pLF999

The E2 gene deleted for its transmembrane and carboxy-terminal domains is synthesized by a PCR reaction from the pLF1022 template (Example 6.2.1) and with the aid of the following oligonucleotide pair:

LF045 (41 mer) (SEQ ID NO 41)

5' CATGACGCGGCCGCCCTATGACCACCACAGCTTTCCTAATC 3' and

LF046 (36 mer) (SEQ ID NO 42)

5' CATGACAGATCTTTATATGAACTCTGAGAAGTAGTC 3'.

The DNA fragment of about 1140 bp obtained by digesting the PCR product with NotI and BglII is ligated with a fragment of 5642 bp resulting from the digestion of

pLF999 (Example 2) with NotI and BglII in order to generate the plasmid pLF1023 (about 6787 bp).

The E2 gene of BVDV-2 strain 890 thus modified (β-globin tPA-E2 Δ[TM+Cter]) encodes a protein of 405 amino acids.

6.2 Plasmids encoding the various forms of E0 of the type 2 - 890 strain

6.2.1 pLF 1030: E0 gene (native form) cloned into the vector pVR1012

The cDNA of the E0 gene of the 890 strain is synthesized from the corresponding viral RNA with the aid of the LF065 primer and amplified by a PCR reaction with the aid of the following oligonucleotide pair:

LF064 (39 mer) (SEQ ID NO 43)

5' CATACCGTCGACATGAGAAAGAAATTGGAGAAGGCACTG 3' and

LF065 (39 mer) (SEQ ID NO 44)

5' CATACCGGATCCTCATGCTGCATGAGCACCAAACCATGC 3'.

The DNA fragment of about 768 bp obtained by digesting the PCR product with SalI and BamHI is ligated with a fragment of 4866 bp resulting from the digestion of pVR1012 (Example 2) with SalI and BamHI in order to generate the plasmid pLF1030 (about 5639 bp). The E0 gene of BVDV-2 strain 890 encodes a protein of 253 amino acids.

An ATG codon is introduced into the sequence of the oligonucleotide LF064 so as to allow the initiation of the translation of the corresponding recombinant E0 polypeptide.

6.2.2 pLF1031: E0 gene, (β-globin tPA-E0) form, cloned into the vector pLF999.

The E0 gene is synthesized by a PCR reaction from the pLF1030 template (Example 6.2.1.) and with the aid of the following oligonucleotide pair:

LF066 (42 mer) (SEQ ID NO 45)

5' CATGACGCGGCCGCTATGAGAAAGAAATTGGAGAAGGCACTG 3' and

LF067 (39 mer) (SEQ ID NO 46)

5' CATACCAGATCTTCATGCTGCATGAGCACCAAACCATGC 3'.

The DNA fragment of about 770 bp obtained by digesting the PCR product with NotI and BglII is ligated with a fragment of 5642 bp resulting from the digestion of pLF999 (Example 2) with NotI and BglII in order to generate the plasmid pLF1031 (about 6417 bp).

The E0 gene of BVDV-2 strain 890 thus modified (β-globin tPA-E0) encodes a protein of 283 amino acids.

Example 7: Plasmids encoding the various forms of the bovine parainfluenza virus type 3 (bPI-3) antigens

The genes encoding the hemagglutinin-neuraminidase (HN) and fusion (F) antigens of the bPI-3 virus are obtained by RT-PCR from the viral RNA of the Reisinger SF-4 strain (accessible from ATCC under the number VR-281).

7.1 Plasmids encoding the various forms of HN of the bPI-3 SF-4 strain

7.1.1 pLF1024: HN gene (native form) cloned into the vector pVR1012

The cDNA of the HN gene of the SF-4 strain is synthesized from the corresponding viral RNA with the aid of the primer LF048 and amplified by a PCR reaction with the aid of the following oligonucleotide pair:

LF047 (39 mer) (SEQ ID NO 47)

5' CATATCGTCGACATGGAATATTGGAAACACACAAACAGC 3' and
LF048 (38 mer) (SEQ ID NO 48)
5' CATGACGATATCTAGCTGCAGTTTTTCGGAACTTCTGT 3'.

The DNA fragment of 1726 bp obtained by digesting the PCR product with SalI and EcoRV is ligated with a fragment of 4896 bp resulting from the digestion of pVR1012 (Example 2) with SalI and EcoRV in order to generate the plasmid pLF1024 (about 6619 bp).

The bPI-3 HN gene encodes a protein of 572 amino acids.

7.1.2 pLF1025: HN gene, (β-globin tPA-E2 Δ[TM]) form, cloned into the vector pLF999

The HN gene deleted for its transmembrane domain is synthesized by a PCR reaction from the pLF1024 template (Example 7.1.1) with the aid of the following oligonucleotide pair:
LF058 (33 mer) (SEQ ID NO 49)
5' CATACTGCGGCCGCTTTAATTCAAGAGAACAAT 3' and
LF059 (35 mer) (SEQ ID NO 50)
5' CATATCGATATCTAGCTGCAGTTTTTCGGAACTTC 3'.

The DNA fragment of 1566 bp obtained by digesting the PCR product with NotI and EcoRV is ligated with a fragment of 5663 bp resulting from the digestion of pLF999 (Example 2) with NotI and EcoRV in order to generate the plasmid pLF1025 (about 7229 bp).

The bPI-3 HN gene thus modified (β-globin tPA-E2 Δ[TM]) encodes a protein of 548 amino acids.

7.2 Plasmids encoding the various forms of F of the bPI-3 SF-4 strain

7.2.1 pLF1026: F gene (native form) cloned into the vector pVR1012

The cDNA of the F gene of strain SF-4 is synthesized from the corresponding viral RNA with the aid of the primer LF061 and amplified by a PCR reaction with the aid of the following oligonucleotide pair:

LF060 (36 mer) (SEQ ID NO 51)

5' CATATCGTCGACATGATCATCACAAACACAATCATA 3' and

LF061 (36 mer) (SEQ ID NO 52)

5' CATGACCAGATCTTATTGTCTATTTGTCAGTATATA 3'.

The DNA fragment of 1628 bp obtained by digesting the PCR product with SalI and BglII is ligated with a fragment of 4860 bp resulting from the digestion of pVR1012 (Example 2) with SalI and BglII in order to generate the plasmid pLF1026 (about 6488 bp).

The bPI-3 F gene encodes a protein of 550 amino acids.

7.2.2 pLF1027: F gene, (β-globin tPA-F Δ[TM+Cter]) form, cloned into the vector pLF999

The F gene deleted for its transmembrane and C-terminal domains is synthesized by a PCR reaction from the pLR1026 template (Example 7.2.1) and with the aid of the following oligonucleotide pair:

LF062 (42 mer) (SEQ ID NO 53)

5' CATACTGCGGCCGCTCAAATAGACATAACAAAACTGCAACGT 3' and

LF063 (41 mer) (SEQ ID NO 54)

5' CATATCGATATCTATGCACTAGATTGATACCAACTTCCAAC 3'.

The DNA fragment of 1434 bp obtained by digesting the PCR product with NotI and EcoRV is ligated with a fragment of 5663 bp resulting from the digestion of pLF999

(Example 2) with NotI and EcoRV in order to generate the plasmid pLF1027 (about 7097 bp).

The bPI-3 F gene thus modified (β-globin tPA-F Δ[TM+Cter]) encodes a protein of 504 amino acids.

Example 8: Plasmids encoding the various forms of the pseudorabies virus (PRV) antigens

The genes encoding the PRV glycoproteins gB, gC and gD are obtained by PCR from the viral DNA of the NIA3 strain (M. Rivière *et al.* J. Virol. **66**, 3424-3434; A. Baskerville *et al.* The Veterinary Bulletin, 1973, **43 No. 9**). Mutants of the PRV NIA3 strain may also be used and are described in US-A-4,680,176 and deposited with the Collection Nationale de Cultures de Microorganismes (CNCM), Institut Pasteur, Paris, France, under the references I-351 and I-352.

8.1. Plasmids encoding the various forms of PRV-gB

8.1.1. pSB101: gB gene (native form) cloned into the vector pVR1012

The gB gene of the PRV NIA3 strain is amplified by PCR using the viral DNA as template and with the aid of the following primers:

SB201 (36 mer) (SEQ ID NO 55)
5' TTTTAAGATATCATGCCCGCTGGTGGCGGTCTTTGG 3' and
SB202 (39 mer) (SEQ ID NO 56)
5' TTTTAAGGATCCCTACAGGGCGTCGGGGTCCTCGCTCTC 3'.

The amplification product (2766 bp) is digested with the enzymes EcoRV and BamHI and cloned into the vector pVR1012 (Example 2) previously digested with EcoRV and

BamHI, generating the plasmid pSB101, having a size of about 7631 bp.

The PRV gB gene encodes a glycoprotein of 913 amino acids.

8.1.2 pSB102: gB gene (Δ[TM-Cter] form) cloned into the vector pVR1012

The truncated form of the gB gene of the PRV NIA3 strain is amplified by PCR using the viral DNA as template and with the aid of the following primers:

SB201 (SEQ ID NO 55) and

SB203 (39 mer) (SEQ ID NO 57)

5' TTTTAAGGATCCCTAGTGGTCCACCTTGACCACGCGGTC 3'.

The amplification product (2262 bp) is digested with the enzymes EcoRV and BamHI and cloned into the vector pVR1012 (Example 2) previously digested with EcoRV and BamHI, generating the plasmid pSB102, having a size of about 7142 bp.

The truncated form (Δ[TM-Cter]) of the gB gene encodes a glycoprotein of 750 amino acids, containing the extracellular domain of the PRV gB glycoprotein.

8.1.3 pNS009: gB gene (tPA Δ[TM-Cter] form) cloned into the vector pAB110

The tPA Δ[TM-Cter] form of the gB gene of the PRV NIA3 strain is amplified by PCR from the template pSB101 (Example 8.1.1) and with the aid of the following primers:

SB203 (SEQ ID NO 57) and

SB217 (39 mer) (SEQ ID NO 58)

5' AAAATTTCGATATCCACCTCGGCCTCGCCGACGCCCGGG 3'.

The amplification product (2088 bp) is digested with the enzymes EcoRV and BglII and cloned into the vector

pAB110 (Example 2) previously digested with EcoRV and BglII, generating the plasmid pNS009, having a size of about 7127 bp.

The tPA Δ[TM-Cter] form of the gB gene encodes a glycoprotein of 720 amino acids, containing the extracellular domain of the PRV gB glycoprotein.

8.2 Plasmids encoding the various forms of PRV-gC

8.2.1 pSB103: gC gene (native form) cloned into the vector pVR1012

The gC gene of the PRV NIA3 strain is amplified by PCR using the viral DNA as template and with the aid of the following primers:

SB204 (36 mer) (SEQ ID NO 59)

5' TTTTAAGATATCATGGCCTCGCTCGCGCGTGCGATG 3' and

SB205 (37 mer) (SEQ ID NO 60)

5' TTTTAAAGATCTTTAAGGCCCCGCCTGGCGGTAGTAG 3'.

The amplification product (1452 bp) is digested with the enzymes EcoRV and BglII and cloned into the vector pVR1012 (Example 2) previously digested with EcoRV and BglII, generating the plasmid pSB103, having a size of about 6323 bp.

The PRV gC gene encodes a glycoprotein of 479 amino acids.

8.2.2 pSB104: gC gene (Δ[TM-Cter] form) cloned into the vector pVR1012

The truncated form of the gC gene of the PRV NIA3 strain is amplified by PCR using the viral DNA as template and with the aid of the following primers:

SB204 (SEQ ID NO 59) and

SB206 (36 mer) (SEQ ID NO 61)

5' TTTTAAAGATCTTTAGGGGGAGGCGTCGTAGCGCTG 3'.

The amplification product (1332 bp) is digested with the enzymes EcoRV and BglII and cloned into the vector pVR1012 (Example 2) previously digested with EcoRV and BglII, generating the plasmid pSB104, having a size of about 6206 bp.

The truncated form (Δ[TM-Cter]) of the gC gene encodes a glycoprotein of 440 amino acids, containing the extracellular domain of the PRV gC glycoprotein.

8.2.3 pNS012: gC gene (tPA Δ[TM-Cter] form) cloned into the vector pAB110

The tPA Δ[TM-Cter] form of the gC gene of the PRV NIA3 strain is amplified by PCR from the template pSB103 (Example 8.2.1) and with the aid of the following primers:

SB206 (SEQ ID NO 61) and

SB218 (39 mer) (SEQ ID NO 62)

5' AAAATTTCGATATCCACGGCGCTCGGCACGACGCCCAAC 3'.

The amplification product (1270 bp) is digested with the enzymes EcoRV and BglII and cloned into the vector pAB110 (Example 2) previously digested with EcoRV and BglII, generating the plasmid pNS012, having a size of about 6311 bp.

The tPA Δ[TM-Cter] form of the gC gene encodes a glycoprotein of 448 amino acids, containing the extracellular domain of the PRV gC glycoprotein.

8.3 Plasmids encoding the various forms of PRV-gD

8.3.1 pSB105: gD gene (native form) cloned into the vector pVR1012

The gD gene of the PRV NIA3 strain is amplified by PCR using the viral DNA as template and with the aid of the following primers:

SB207 (36 mer) (SEQ ID NO 63)

5' AATTTTGATATCATGCTGCTCGCAGCGCTATTGGCG 3' and

SB208 (36 mer) (SEQ ID NO 64)

5' AATTTTGGATCCCTACGGACCGGGCTGCGCTTTTAG 3'.

The amplification product (1227 bp) is digested with the enzymes EcoRV and BamHI and cloned into the vector pVR1012 (Example 2) previously digested with EcoRV and BamHI, generating the plasmid pSB105, having a size of about 6104 bp.

The PRV gD gene encodes a glycoprotein of 404 amino acids.

8.3.2 pSB106: gD gene (Δ[TM-Cter] form) cloned into the vector pVR1012

The truncated form of the gD gene of the PRV NIA3 strain is amplified by PCR using the viral DNA as template and with the aid of the following primers:

SB207 (SEQ ID NO 63) and

SB209 (40 mer) (SEQ ID NO 65)

5' AAATTTTGGATCCCTAGCGGTGGCGCGAGACGCCCGGCGC 3'.

The amplification product (1077 bp) is digested with the enzymes EcoRV and BamHI and cloned into the vector pVR1012 (Example 2) previously digested with EcoRV and BamHI, generating the plasmid pSB106 having a size of about 5957 bp.

The truncated form (Δ[TM-Cter]) of the gD gene encodes a glycoprotein of 355 amino acids, containing the extracellular domain of the PRV gD glycoprotein.

8.3.3 pPB238: gD gene (tPA Δ[TM-Cter] form) cloned into the vector pAB110

The tPA Δ[TM-Cter] form of the gD gene of the PRV NIA3 strain is amplified by PCR from the template pSB105 (Example 8.3.1) and with the aid of the following primers:

SB209 (SEQ ID NO 65) and

SB219 (39 mer) (SEQ ID NO 66)

5' AAAATTTCGATATCCACCTTCCCCCCGCCCGCGTACCCG 3'.

The amplification product (1015 bp) is digested with the enzymes EcoRV and BamHI and cloned into the vector pAB110 (Example 2) previously digested with EcoRV and BglII, generating the plasmid pPB238, having a size of about 6056 bp.

The tPA Δ[TM-Cter] form of the gD gene encodes the glycoprotein of 363 amino acids, containing the extracellular domain of the PRV gD glycoprotein.

Example 9: Plasmids encoding the various forms of the porcine reproductive respiratory syndrome virus (PRRSV), strain Lelystad, antigens

The genes encoding the PRRSV ORF3, ORF5 and ORF6 proteins are obtained by RT-PCR from the viral RNA of the Lelystad strain (J. Meulenberg *et al.* Virology, 1993, **19**, 62-72; WO-A-92-21375), deposited June 5, 1991 with the Collection Nationale de Cultures de Microorganismes (CNCM), Institut Pasteur, Paris, France, under the reference I-1102.

9.1 Plasmids encoding the various forms of the PRRSV Lelystad strain ORF3

9.1.1 pLF1009: ORF3 gene (native form) cloned into the vector pVR1012

The cDNA of the ORF3 gene of the Lelystad strain is synthesized from the corresponding viral RNA with the aid of the primer LF028 and amplified by a PCR reaction with the aid of the following oligonucleotide pair:

LF027 (30 mer) (SEQ ID NO 67)

5' CACTACGATATCATGGCTCATCAGTGTGCA 3' and

LF028 (30 mer) (SEQ ID NO 68)

5' CACTACAGATCTTTATCGTGATGTACTGGG 3'.

The DNA fragment of 802 bp obtained by digesting the PCR product with EcoRV and BglIII is ligated with a fragment of 4879 bp resulting from the digestion of pVR1012 (Example 2) with EcoRV and BglIII in order to generate the plasmid pLF1009 having a size of about 5681 bp.

The PRRSV Lelystad ORF3 gene encodes a protein of 265 amino acids.

9.2 Plasmids encoding the various forms of the PRRSV Lelystad strain ORF5

9.2.1 pLF1011: ORF5 gene (native form) cloned into the vector pVR1012

The cDNA of the ORF5 gene of the Lelystad strain is synthesized from the corresponding viral RNA with the aid of the primer LF020 and amplified by a PCR reaction with the aid of the following oligonucleotide pair:

LF019 (30 mer) (SEQ ID NO 69)

5' CTCACCGTCGACATGAGATGTTCTCACAAA 3' and

LF020 (30 mer) (SEQ ID NO 70)

5' CTCACCTCTAGACTAGGCCTCCCATTGCTC 3'.

The DNA fragment of 802 bp obtained by digesting the PCR product with SalI and XbaI is ligated with a fragment of 4879 bp resulting from the digestion of pvR1012 (Example 2) with SalI and XbaI in order to generate the plasmid pLF1011 having a size of about 5681 bp.

The PRRSV Lelystad ORF5 gene encodes a protein of 201 amino acids.

9.2.2 pLF1012: ORF5 gene (truncated form) cloned into the vector pAB110

The ORF5 gene deleted for its transmembrane and carboxy-terminal domains is synthesized by a PCR reaction from the template pLF1011 (Example 9.2.1) with the aid of the following oligonucleotide pair:

LF021 (30 mer) (SEQ ID NO 71)
5' CACCTCGGATCCTTTGCCGATGGCAACGGC 3' and
LF022 (33 mer) (SEQ ID NO 72)
5' CACCTCGGATCCTTAGACTTCGGCTTTGCCCAA 3'.

The DNA fragment of 432 bp obtained by digesting the PCR product with BamHI is ligated with a fragment of 5105 bp resulting from the digestion of pAB110 (Example 2) with BamHI in order to generate the plasmid pLF1012 having a size of about 5537 bp.

The PRRSV Lelystad ORF5 gene thus modified (tPA Δ[TM+Cter]) encodes a protein of 168 amino acids.

9.3 Plasmids encoding the various forms of the PRRSV Lelystad strain ORF6

9.3.1 pLF1013: ORF6 gene (native form) cloned into the vector pVR1012

The cDNA of the ORF6 gene of the Lelystad strain is synthesized from the corresponding viral RNA with the aid of the primer LF024 and amplified by a PCR reaction with the aid of the following oligonucleotide pair:

LF023 (30 mer) (SEQ ID NO 73)

5' CACTCAGTCGACATGGGAGGCCTAGACGAT 3' and

LF024 (30 mer) (SEQ ID NO 74)

5' CACTCATCTAGATTACCGGCCATACTTGAC 3'.

The DNA fragment of 528 bp obtained by digesting the PCR product with SalI and XbaI is ligated with the fragment of 4881 bp resulting in the digestion of pVR1012 (Example 2) with SalI and XbaI in order to generate the plasmid pLF1013 having a size of about 5409 bp.

The PRRSV Lelystad ORF6 gene encodes a protein of 173 amino acids.

9.3.2 pLF1014: ORF6 gene (truncated form) cloned into the vector pAB110

The ORF6 gene deleted for its transmembrane and carboxy-terminal domains is synthesized by a PCR reaction from the template pLF1013 (Example 9.3.1) with the aid of the following oligonucleotide pair:

LF025 (30 mer) (SEQ ID NO 75)

5' CACTACGGATCCGTGTCACGCGGCCGACTC 3' and

LF026 (33 mer) (SEQ ID NO 76)

5' CACTACGGATCCTTAAACAGCTCGTTTGCCGCC 3'.

The DNA fragment of 390 bp obtained by digesting the PCR product with BamHI is ligated with a fragment of 5105 bp resulting from the digestion of pAB110 (Example 2) with BamHI in order to generate the plasmid pLF1014 having a size of about 5495 bp.

The PRRSV Lelystad ORF6 gene thus modified (tPA Δ[TM+Cter]) encodes a protein of 154 amino acids.

Example 10: Plasmids encoding the various forms of the porcine reproductive respiratory syndrome virus (PRRSV), American strain ATCC VR-2332, antigens

The genes encoding the PRRSV virus ORF3, ORF5 and ORF6 proteins are obtained by RT-PCR from the viral RNA of the American strain (M. Murtaugh *et al.* Arch Virol. 1995, **140**, 1451-1460), deposited with the ATCC under the number VR-2332.

10.1 Plasmids encoding the various forms of PRRSV VR-2332 strain ORF3

10.1.1 pLF1015: ORF3 gene (native form) cloned into the vector pVR1012

The cDNA of the ORF3 gene of the VR-2332 strain is synthesized from the corresponding viral RNA with the aid of the primer LF038 and amplified by a PCR reaction with the aid of the following oligonucleotide pair :

LF037 (30 mer) (SEQ ID NO 77)

5' CACTACGATATCATGGTTAATAGCTGTACA 3' and

LF038 (30 mer) (SEQ ID NO 78)

5' CACTACTCTAGACTATCGCCGTACGGCACT 3'.

The DNA fragment of 769 bp obtained by digesting the PCR product with EcoRV and XbaI is ligated with a fragment of 4900 bp resulting from the digestion of pVR1012 (Example 2) with EcoRV and BglII in order to generate the plasmid pLF1015 having a size of about 5669 bp.

The PRRSV strain VR-2332 ORF3 gene encodes a protein of 254 amino acids.

10.2 Plasmids encoding the various forms of the PRRSV VR-2332 strain ORF5

10.2.1 pLF1017: ORF5 gene (native form) cloned into the vector pVR1012

The cDNA of the ORF5 gene of the VR-2332 strain is synthesized from the corresponding viral RNA with the aid of the primer LF030 and amplified by a PCR reaction with the aid of the following oligonucleotide pair:

LF029 (30 mer) (SEQ ID NO 79)

5' CACTACGATATCATGTTGGAGAAATGCTTG 3' and

LF030 (30 mer) (SEQ ID NO 80)

5' CACTACAGATCTCTAAGGACGACCCCATTG 3'.

The DNA fragment of 607 bp obtained by digesting the PCR product with EcoRV and BglII is ligated with a fragment of 4879 bp resulting from the digestion of pVR1012 (Example 2) with EcoRV and BglII in order to generate the plasmid pLF1017 having a size of about 5486 bp.

The PRRSV strain VR-2332 ORF5 gene encodes a protein of 200 amino acids.

10.2.2 pLF1018: ORF5 gene (truncated form) cloned into the vector pAB110

The ORF5 gene deleted for its transmembrane and carboxy-terminal domains is synthesized by a PCR reaction from the template pLF1017 (Example 10.2.1) with the aid of the following oligonucleotide pair:

LF031 (33 mer) (SEQ ID NO 81)

5' CACTACGGATCCGCCAGCAACGACAGCAGCTCC 3' and

LF032 (33 mer) (SEQ ID NO 82)

5' CACTACGGATCCTTAGACCTCAACTTTGCCCCT 3'.

The DNA fragment of 426 bp obtained by digesting the PCR product with BamHI is ligated with a fragment of 5105 bp resulting from the digestion of pAB110 (Example 2) with BamHI in order to generate the plasmid pLF1018 having a size of about 5531 bp.

The PRRSV strain VR-2332 ORF5 gene thus modified (tPA Δ[TM+Cter]) encodes a protein of 166 amino acids.

10.3 Plasmids encoding the various forms of the PRRSV VR-2332 strain ORF6

10.3.1 pLF1019: ORF6 gene (native form) cloned into the vector pVR1012

The cDNA of the ORF6 gene of the VR-2332 strain is synthesized from the corresponding viral RNA with the aid of the primer LF034 and amplified by a PCR reaction with the aid of the following oligonucleotide pair:

LF033 (33 mer) (SEQ ID NO 83)

5' CACATCCTGCAGATGGGGTCGTCCTTAGATGAC 3' and

LF034 (30 mer) (SEQ ID NO 84)

5' CACATCTCTAGATTATTTGGCATATTTGAC 3'.

The DNA fragment of 527 bp obtained by digesting the PCR product with PstI and XbaI is ligated with a fragment of 4871 bp resulting from the digestion of pVR1012 (Example 2) with PstI and XbaI in order to generate the plasmid pLF1019 having a size of about 5398 bp.

The PRRSV strain VR-2332 ORF6 gene encodes a protein of 174 amino acids.

10.3.2 pLF1016: ORF6 gene (truncated form) cloned into the vector pAB110

The ORF6 gene deleted for its transmembrane and carboxy-terminal domains is synthesized by a PCR reaction from the template pLF1019 (Example 10.3.1) with the aid of the following oligonucleotide pair:

LF035 (30 mer) (SEQ ID NO 85)

5' CACTACGGATCCGTGAGTCGCGGCCGACTG 3' and

LF036 (33 mer) (SEQ ID NO 86)

5' CACTACGGATCCTTAAACAGCTTTTCTGCCACC 3'.

The DNA fragment of 390 bp obtained by digesting the PCR product with BamHI is ligated with a fragment of 5105 bp resulting from the digestion of pAB110 (Example 2) with BamHI in order to generate the plasmid pLF1016 having a size of about 5459 bp.

The PRRSV strain VR-2332 ORF6 gene thus modified (tPA Δ[TM+Cter]) encodes a protein of 154 amino acids.

Example 11: Plasmids encoding the various forms of the swine influenza virus (SIV), strain H1N1, antigens

The genes encoding the hemagglutinin (HA) and neuraminidase (NA) antigens of the swine influenza virus type H1N1 are obtained by RT-PCR from the viral RNA of the "SW" H1N1 strain. Strains are available from the Virology Research Center, Armand-Frappier Institute, University of Quebec, Laval, Canada (D.S. Arora *et al.*, Virus Genes, 1997, **14**, 251-254). See also G.W. Both *et al.,* Proc. Natl. Acad. Sci. USA, 1983, **80**, 6996-7000.

11.1 Plasmids encoding the various forms of SIV H1N1 strain HA

11.1.1 pLF1001: HA gene (native form) cloned into the vector pVR1012

The cDNA of the HA gene of the H1N1 strain is synthesized from the corresponding viral RNA with the aid of the primer LF004 and amplified by a PCR reaction with the aid of the following oligonucleotide pair:

LF003 (30 mer) (SEQ ID NO 87)

5' CTCCATGATATCATGGAAGCAAAACTATTC 3' and

LF004 (30 mer) (SEQ ID NO 88)

5' CTCCATCAGATCTTAAATGCATATTCTGCA 3'.

The DNA fragment of 1705 bp obtained by digesting the PCR product with EcoRV and BglII is ligated with the fragment of 4879 bp resulting from the digestion of pVR1012 (Example 2) with EcoRV and BglII in order to generate the plasmid pLF1001 having a size of about 6584 bp.

The SIV H1N1 HA gene encodes a protein of 566 amino acids.

11.1.2 pLF1002: HA gene (modified form) cloned into the vector pLF999

The HA gene deleted for its transmembrane and carboxy-terminal domains is synthesized by a PCR reaction from the template pLF1001 (Example 11.1.1) with the aid of the following oligonucleotide pair:

LF005 (30 mer) (SEQ ID NO 89)

5' TCCGCGGCCGCACATGCTAACAATTCCACA 3' and

LF006 (32 mer) (SEQ ID NO 90)

5' TCCGCGGCCGCTTACATTGATTCTAGTTTCAC 3'.

The DNA fragment of 1515 bp obtained by digesting the PCR product with NotI is ligated with a fragment of 5678 bp resulting from the digestion of pLF999 (Example 2) with NotI in order to generate the plasmid pLF1002 having a size of 7193 bp.

The SIV H1N1 HA gene thus modified (intron II of the rabit β-globin gene, tPA, Δ[TM+Cter]) encodes a protein of 530 amino acids.

11.2 Plasmids encoding the various forms of the SIV H1N1 strain NA

11.2.1 pLF1003: NA gene (native form) cloned into the vector pVR1012

The cDNA of the NA gene of the H1N1 strain is synthesized from the corresponding viral RNA with the aid of the primer LF008 and amplified by a PCR reaction with the aid of the following oligonucleotide pair:

LF007 (30 mer) (SEQ ID NO 91)

5' CACCTGGTCGACATGAATCCAAATCAGAAG 3' and

LF008 (30 mer) (SEQ ID NO 92)

5' CACCTGTCTAGACTACTTGTCAATGGTGAA 3'.

The DNA fragment of 1416 bp obtained by digesting the PCR product with SalI and XbaI is ligated with a fragment of 4881 bp resulting from the digestion of pVR1012 (Example 2) with SalI and XbaI in order to generate the plasmid pLF1003 having a size of about 6297 bp.

The SIV H1N1 NA gene encodes a protein of 469 amino acids.

11.2.2 pLF1004: NA gene (modified form) cloned into the vector pLF999

The NA gene deleted for its transmembrane and carboxy-terminal domains is synthesized by a PCR reaction from the template pLF1003 with the aid of the following oligonucleotide pair:

LF009 (31 mer) (SEQ ID NO 93)

5' CACTACGAATTCACAAATTGGGAATCAAAAT 3' and

LF010 (30 mer) (SEQ ID NO 94)

5' AATTTGTGAATTCGCGGCCGCGGATCCGGT 3'.

The DNA fragment of 1207 bp obtained by digesting the PCR product with EcoRI is ligated with a fragment of 5678 bp resulting from the digestion of pLF999 (Example 2) with EcoRI in order to generate the plasmid pLF1004 having a size of about 6885 bp.

The SIV H1N1 NA gene thus modified (intron II of the rabbit β-globin gene, tPA, Δ[TM+Cter]) encodes a protein of 431 amino acids.

Example 12: Plasmids encoding the various forms of the swine influenza virus (SIV), strain H3N2, antigens

The genes encoding the HA and NA antigens of the type H3N2 swine influenza virus are obtained by RT-PCR from the viral RNA of the "Côtes du Nord 1987" (cdn87) strain referenced by the World Health Organization (WHO) and available from the National Influenza Reference Center, Virology Laboratory, 8 avenue Rockfeller, 69008 Lyon, France.

12.1 Plasmids encoding the various forms of the SIV H3N2 strain HA

12.1.1 pLF1005: HA gene (native form) cloned into the vector pVR1012

The cDNA of the HA gene of the H3N2 strain is synthesized from the corresponding viral RNA with the aid of the primer LF012 and amplified by a PCR reaction with the aid of the following oligonucleotide pair:

LF011 (30 mer) (SEQ ID NO 95)

5' CTGCACGTCGACATGAAGACTGTCATTGCC 3' and

LF012 (24 mer)(SEQ ID NO 96)

5' GATATCTCAGATGCAAATGTTGCA 3'.

The DNA fragment of 1709 bp obtained by digesting the PCR product with EcoRV and SalI is ligated with a fragment of 4893 bp resulting from the digestion of pVR1012 (Example 2) with EcoRV and SalI in order to generate the plasmid pLF1005 having a size of about 6602 bp.

The SIV H3N2 HA gene encodes a protein of 566 amino acids.

12.1.2 pLF1006: HA gene (modified form) cloned into the vector pLF999

The HA gene deleted for its transmembrane and carboxy-terminal domains is synthesized by a PCR reaction from the template pLF1005 (Example 12.1.1) with the aid of the following oligonucleotide pair:

LF013 (33 mer) (SEQ ID NO 97)

5' CACCGCGGATCCCTTCCAGAAAATGGCAGCACA 3' and

LF014 (33 mer) (SEQ ID NO 98)

5' CACCGCGGATCCTTAGTCTTTGTATCCCGACTT 3'.

The DNA fragment of 1542 bp obtained by digesting the PCR product with BamHI is ligated with a fragment of 5678 bp resulting from the digestion of pLF999 (Example 2) with BamHI in order to generate the plasmid pLF1006 having a size of about 7220 bp.

The SIV H3N2 HA gene thus modified (intron II of the rabbit β-globin gene, tPA, Δ[TM+Cter]) encodes a protein of 538 amino acids.

12.2 Plasmids encoding the various forms of the SIV H3N2 strain NA

12.2.1 pLF1007: NA gene (native form) cloned into the vector pVR1012

The cDNA of the NA gene of the H3N2 strain is synthesized from the corresponding viral RNA with the aid of the primer LF016 and amplified by a PCR reaction with the aid of the following oligonucleotide pair:

LF015 (30 mer) (SEQ ID NO 99)

5' CACTCAGATATCATGAATCCAAAGCAAAAG 3' and

LF016 (30 mer) (SEQ ID NO 100)

5' CACTCATCTAGATTATATAGGCATGAGATC 3'.

The DNA fragment of 1414 bp obtained by digesting the PCR product with EcoRV and XbaI is ligated with a fragment of 4900 bp resulting from the digestion of pVR1012 (Example 2) with EcoRV and XbaI in order to generate the plasmid pLF1007 having a size of about 6314 bp.

The SIV H3N2 NA gene encodes a protein of 469 amino acids.

12.2.2 pLF1008: NA gene (modified form) cloned into the vector pLF999

The NA gene deleted for its transmembrane and carboxy-terminal domains is synthesized by a PCR reaction from the template pLF1005 (Example 12.2.1) with the aid of the following oligonucleotide pair:

LF017 (33 mer) (SEQ ID NO 101)

5' CACTACGGATCCTTCAAGCAATATGAGTGCGAC 3' and

LF018 (33 mer) (SEQ ID NO 102)

5' CACTACGGATCCTTATGAAGTCCACCATACTCT 3'.

The DNA fragment of 1221 bp obtained by digesting the PCR product with BamHI is ligated with a fragment of 5678 bp resulting from the digestion of pLF999 (Example 2)

with BamHI in order to generate the plasmid pLF1008 having a size of about 6899 bp.

The SIV H3N2 NA gene thus modified (intron II of the rabbit β-globin gene, tPA, Δ[TM+Cter]) encodes a protein of 431 amino acids.

Example 13: Plasmid encoding bovine GM-CSF

The cDNA of the bovine GM-CSF gene is synthesized from the cellular RNA of bovine blood mononucleated cells with the aid of the primer LF065 and amplified by a PCR reaction with the aid of the following oligonucleotide pair:

LF054 (36 mer) (SEQ ID NO 103)
5' CATATCGTCGACATGTGGCTGCAGAACCTGCTTCTC 3' and
LF055 (34 mer) (SEQ ID NO 104)
5' CATGACCAGATCTTCACTTCTGGGCTGGTTCCCA 3'.

The DNA fragment of 437 bp obtained by digesting the PCR product with SalI and BglII is ligated with a fragment of 4860 bp resulting from the digestion of pVR1012 (Example 2) with SalI and BglII in order to generate the plasmid pLF1032 (about 5297 bp). The bovine GM-CSF gene encodes a protein of 143 amino acids.

Example 14: Plasmid encoding porcine GM-CSF

The cDNA of the porcine GM-CSF gene is synthesized from the cellular RNA of porcine blood mononucleated cells with the aid of the primer LF067 and amplified by a PCR reaction with the aid of the following oligonucleotide pair:

LF056 (36 mer) (SEQ ID NO 105)
5' CATATCGTCGACATGTGGCTGCAGAACCTGCTTCTC 3' and
LF057 (37 mer) (SEQ ID NO 106)

5' CATGACCAGATCTTCACTTCTGGGCTGGTTCCCAGCA 3'.

The DNA fragment of 440 bp obtained by digesting the PCR product with SalI and BglII is ligated with a fragment of 4860 bp resulting from the digestion of pVR1012 (Example 2) with SalI and BglII in order to generate the plasmid pLF1033 (about 5300 bp). The porcine GM-CSF gene encodes a protein of 144 amino acids.

Example 15: Formulation of the vaccinal plasmids

The DNA solution containing one or more plasmids according to Examples 3 to 14 is concentrated by ethanolic precipitation as described in Sambrook *et al.* (1989). The DNA pellet is taken up in a 0.9% NaCl solution so as to obtain a concentration of 1 mg/ml. A 0.75 mM DMRIE-DOPE solution is prepared by taking up a lyophilisate of DMRIE-DOPE with an appropriate volume of sterile H_2O.

The formation of the plasmid DNA-lipid complexes is achieved by diluting, in equal parts, the 0.75 mM DMRIE-DOPE solution with the DNA solution at 1 mg/ml in 0.9% NaCl. The DNA solution is gradually introduced, with the aid of a seamed 26G needle, along the wall of the vial containing the cationic lipid solution so as to avoid the formation of foam. Gentle shaking is carried out as soon as the two solutions have been mixed. A composition comprising 0.375 mM of DMRIE-DOPE and 500 µg/ml of plasmid is finally obtained.

It is desirable for all the solutions used to be at room temperature for all the operations described above. The DNA/DMRIE-DOPE complex formation is allowed to take place at room temperature for 30 minutes before immunizing the animals.

Example 16: Immunization of bovines against BHV-1

12 bovines are randomized into 3 groups of 4 s.

Group 1 constitutes the control animal group.

A mixture of vaccinal plasmids pPB281 (encoding BHV-1 gB in a Δ[TM-Cter] form, Example 3.1.2), pPB292 (encoding BHV-1 gC in a Δ[TM-Cter] form, Example 3.2.2) and pPB284 (encoding BHV-1 gD in a Δ[TM-Cter] form, Example 3.3.2) is administered to the animals of Group 2.

The same mixture as that in Group 2, but formulated with DMRIE-DOPE as is described in Example 15, is administered to the animals of Group 3.

An injection of 10 ml, by the intramuscular route, is performed on each bovine with the aid of syringes equipped with needle, and is repeated 21 days later. The total mass of each plasmid used during each immunization is 1500 μg.

Persons skilled in the art possess the necessary competence to adjust the volume or the concentration according to the plasmid dose required.

Monitoring of the serological response induced by the two mixtures of vaccine plasmids expressing the BHV-1 gB, gC and gD antigens is carried out over a period of 35 days after the first vaccination.

The results are presented in the table which follows:

Plasmids	Formulation	Antigens	Dose	SN at D28	SN at D35
control	---	---	---	0.2 +/- 0.0	0.2 +/-0.0
pPB281 pPB292 pPB294	---	gB Δ[TM-Cter] gC Δ[TM-Cter] gD Δ[TM-Cter]	1500 µg 1500 µg 1500 µg	1.0 +/- 0.5	1.2 +/- 0.8
pPB281 pPB292 pPB294	DMRIE-DOPE	gB Δ[TM-Cter] gC Δ[TM-Cter] gD Δ[TM-Cter]	1500 µg 1500 µg 1500 µg	2.1 +/- 0.6	2.7 +/- 0.6

Example 17: Immunization of pigs against PRV

15 pigs, about 7 weeks old, are randomized into 3 groups of 5 animals.

Group 1 constitutes the control animal group.

A mixture of vaccinal plasmids pNS009 (encoding PRV gB in a tPA Δ[TM-Cter] form, Example 8.1.3), pNS012 (encoding PRV gC in a tPA Δ[TM-Cter] form, Example 8.2.3) and pPB238 (encoding PRV gD in a tPA Δ[TM-Cter] form, Example 8.3.3) is administered to the animals of Group 2.

The same mixture as that in Group 3 but formulated with DMRIE-DOPE as is described in Example 15 is administered to the animals of Group 4 so as to obtain a final DMRIE-DOPE concentration of 0.0535 mM.

350 µg of each plasmid necessary for these vaccination protocols are mixed in a final volume of 14 ml.

An injection of 2 ml, by the intramuscular route, is performed with the aid of syringes equipped with needle on each pig, and is repeated 21 days later.

The pigs are challenged at D35 by nasal administration of 2 ml of a solution of PRV strain NIA3 challenge virus in an amount of 1 ml per nostril and having a titre of $10^{7.76}$ $CCID_{50}$ per ml.

Monitoring of the weight (in kg) of each animal is carried out over a period of 42 days after the first vaccination.

The relative weight gain (G7) is calculated for each animal during the 7 days period which immediately follows the challenge. It is the difference between the weight at day 7 (D7) and that at challenge day (D0), divided by the weight at challenge day, and daily expressed as a percentage:

(weight at D7 - weight at D0) . 100 / (weight at D0 . 7)

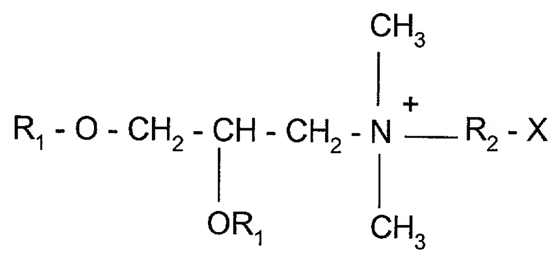
ΔG7 is the difference between the mean values of relative weight gains of vaccinated animals and controls.

The results are presented in the table which follows:

Plasmids	Formulation	Antigens	Dose	Mean weight at D35	Mean weight at D42	ΔG7
Control	---	---	---	25.3 +/- 6.2	22.0 +/- 5.0	---
pNS009 pNS012 pPB238	---	gB Δ[TM-Cter]tPA gC Δ[TM-Cter]tPA gD Δ[TM-Cter]tPA	350 μg 350 μg 350 μg	25.3 +/- 4.8	26.1 +/- 4.7	2.46
pNS009 pNS012 pPB238	DMRIE-DOPE	gB Δ[TM-Cter]tPA gC Δ[TM-Cter]tPA gD Δ[TM-Cter]tPA	350 μg 350 μg 350 μg	23.8 +/- 4.5	26.2 +/- 4.9	3.41

The invention shall now be described by the following numbered paragraphs:

1. DNA vaccine or immunogenic or immunological composition against a pathogen affecting farm animals, advantageously and/or particularly bovines or porcines, comprising a plasmid containing a nucleotide sequence encoding an immunogen of a pathogen of the animal species considered, under conditions allowing the *in vivo* expression of this sequence, and a cationic lipid containing a quaternary ammonium salt, of formula



in which R_1 is a saturated or unsaturated linear aliphatic radical having 12 to 18 carbon atoms, R_2 is another aliphatic radical containing 2 or 3 carbon atoms, and X a hydroxyl or amine group, this lipid being preferably DMRIE.

2. Vaccine or immunogenic or immunological composition according to Paragraph 1, wherein it also comprises DOPE.

3. Vaccine or immunogenic or immunological composition according to Paragraph 1, wherein it comprises, in addition, a GM-CSF protein of the animal species considered.

4. Vaccine or immunogenic or immunological composition according to Paragraph 2, wherein it comprises, in addition, a GM-CSF protein of the animal species considered.

5. Vaccine or immunogenic or immunological composition according to Paragraph 1, wherein it comprises, in

addition, an expression vector containing the gene encoding the GM-CSF protein of the animal species considered, under conditions allowing the *in vivo* expression of this sequence.

6. Vaccine or immunogenic or immunological composition according to Paragraph 2, wherein it comprises, in addition, an expression vector containing the gene encoding the GM-CSF protein of the animal species considered, under conditions allowing the *in vivo* expression of this sequence.

7. Vaccine or immunogenic or immunological composition according to Paragraph 5, wherein the expression vector is a plasmid.

8. Vaccine or immunogenic or immunological composition according to Paragraph 6, wherein the expression vector is a plasmid.

9. Vaccine or immunogenic or immunological composition according to Paragraph 1, wherein the nucleotide sequence encoding a pathogen immunogen is the sequence of a gene from which the part encoding the transmembrane domain has been deleted.

10. Vaccine or immunogenic or immunological composition according to Paragraphs 1, wherein the plasmid containing the nucleotide sequence encoding a pathogen immunogen also contains a nucleotide sequence encoding a heterologous signal sequence, preferably a tPA.

11. Vaccine or immunogenic or immunological composition according to Paragraph 1, wherein the plasmid containing the nucleotide sequence encoding a pathogen immunogen also contains a stabilizing intron.

12. Vaccine or immunogenic or immunological composition according to Paragraph 11, wherein the intron is intron II of the rabbit beta-globin gene.

13. Vaccine or immunogenic or immunological composition according to Paragraph 1, wherein it comprises a nucleotide sequence of BHV-1.

14. Vaccine or immunogenic or immunological composition according to Paragraph 13, wherein it comprises the sequence of the gB gene optimized by a signal sequence, in particular that of the tPA signal of human origin, in place of the sequence of the signal peptide of the glycoprotein gB, and/or by the deletion of the DNA fragment encoding the transmembrane domain of gB.

15. Vaccine or immunogenic or immunological composition according to Paragraph 13, wherein it comprises the sequence of the gC gene optimized by a signal sequence, in particular that of the tPA signal of human origin, in place of the sequence of the signal peptide of the glycoprotein gC, and/or by the deletion of the DNA fragment encoding the transmembrane domain of gC.

16. Vaccine or immunogenic or immunological composition according to Paragraph 13, wherein it comprises the sequence of the gD gene optimized by a signal sequence, in particular that of the tPA signal of human origin, in place of the sequence of the signal peptide of the glycoprotein gD, and/or by the deletion of the DNA fragment encoding the transmembrane domain of gD.

17. Vaccine or immunogenic or immunological composition according to Paragraph 13, wherein it comprises DMRIE-DOPE, an expression plasmid encoding the BHV-1 gB antigen optimized by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain and the

contiguous C-terminal part, a second expression plasmid encoding the BHV-1 gC antigen optimized by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain and the contiguous C-terminal part, and a third expression plasmid encoding the BHV-1 gD antigen optimized by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain and the contiguous C-terminal part.

18. Vaccine or immunogenic or immunological composition according to Paragraph 1, wherein it comprises a nucleotide sequence of BRSV.

19. Vaccine or immunogenic or immunological composition according to Paragraph 18, wherein it comprises the sequence of the BRSV F gene optimized by substitution, by a signal sequence, in particular that of the tPA of human origin, of the signal sequence of the F protein of BRSV, and/or by the deletion of the DNA fragment encoding the transmembrane domain of F.

20. Vaccine or immunogenic or immunological composition according to Paragraph 18, wherein it comprises the sequence of the BRSV G gene optimized by substitution, by a signal sequence, in particular that of the tPA of human origin, of the signal sequence of the G glycoprotein of BRSV, and/or by the deletion of the DNA fragment encoding the transmembrane domain of G.

21. Vaccine or immunogenic or immunological composition according to Paragraph 18, wherein it comprises DMRIE-DOPE, an expression plasmid encoding the F antigen of BRSV optimized by the insertion of the signal sequence of the human tPA in place of the signal sequence of F, and by the deletion of the fragment of the nucleotide sequence of F encoding the transmembrane domain and the contiguous

C-terminal part, and a second expression plasmid encoding the G antigen of BRSV optimized by the insertion of the signal sequence of the human tPA in place of the signal sequence of G, and by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain of G and the contiguous C-terminal part.

22. Vaccine or immunogenic or immunological composition according to Paragraph 1, wherein it comprises a nucleotide sequence of BVDV.

23. Vaccine or immunogenic or immunological composition according to Paragraph 22, wherein it comprises the sequence of the BVDV EO gene optimized by the addition of a signal sequence, in particular that of the tPA of human origin, upstream of the nucleotide sequence encoding the EO protein, and/or by the insertion of an intron, in particular intron II of the rabbit beta-globin gene upstream of the nucleotide sequence encoding EO.

24. Vaccine or immunogenic or immunological composition according to Paragraph 22, wherein it comprises the sequence of the E2 gene optimized by the addition of a signal sequence, in particular that of the tPA of human origin, upstream of the nucleotide sequence encoding the E2 protein, and/or by the deletion of the DNA fragment encoding the transmembrane domain of E2, and/or by the insertion of an intron, in particular intron II of the rabbit beta-globin gene upstream of the nucleotide sequence encoding E2.

25. Vaccine or immunogenic or immunological composition according to Paragraph 22, wherein it comprises DMRIE-DOPE, an expression plasmid encoding the EO antigen of BVDV optimized by the insertion of the signal sequence of the human tPA upstream of EO and by the insertion of intron II

of the rabbit beta-globin gene upstream of EO, and a second plasmid encoding the E2 antigen of BVDV optimized by the insertion of the signal sequence of the human tPA upstream of E2, by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain of E2 and by the insertion of intron II of the rabbit beta-globin gene upstream of E2.

26. Vaccine or immunogenic or immunological composition according to Paragraph 1, wherein it comprises a nucleotide sequence of bPI-3.

27. Vaccine or immunogenic or immunological composition according to Paragraph 26, wherein it comprises the sequence of the bPI-3 HN gene optimized by substitution, by a signal sequence, in particular that of the tPA of human origin, of the signal sequence of HN, and/or by the deletion of the DNA fragment encoding the transmembrane domain of HN, and/or by the insertion of an intron, in particular of intron II of the rabbit beta-globin gene upstream of the nucleotide sequence encoding HN.

28. Vaccine or immunogenic or immunological composition according to Paragraph 26, wherein it comprises the sequence of the bPI-3 F gene optimized by substitution, by a signal sequence, in particular that of the tPA of human origin, of the signal sequence of F, and/or by the deletion of the DNA fragment encoding the transmembrane domain of F, and/or by the insertion of an intron, in particular of intron II of the rabbit beta-globin gene upstream of the nucleotide sequence encoding F.

29. Vaccine or immunogenic or immunological composition according to Paragraph 26, wherein it comprises DMRIE-DOPE, an expression plasmid encoding the HN antigen of bPI-3 optimized by the insertion of the signal sequence of the

human tPA in place of the signal sequence of HN, by the deletion of the fragment of the nucleotide sequence of HN encoding the transmembrane domain and the contiguous C-terminal part and by the insertion of intron II of the rabbit beta-globin gene upstream of HN, and a second expression plasmid encoding the F antigen of bPI-3 optimized by the insertion of the signal sequence of the human tPA in place of the signal sequence of F, by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain of F and the contiguous C-terminal part and by the insertion of intron II of the rabbit beta-globin gene upstream of F.

30. Vaccine or immunogenic or immunological composition according to Paragraph 1, wherein it comprises a nucleotide sequence of PRV.

31. Vaccine or immunogenic or immunological composition according to Paragraph 30, wherein it comprises the sequence of the gB gene optimized by substitution, by a signal sequence, in particular that of the tPA signal of human origin, of the sequence of the signal peptide of the gB glycoprotein, and/or by the deletion of the DNA fragment encoding the transmembrane domain of gB.

32. Vaccine or immunogenic or immunological composition according to Paragraph 30, wherein it comprises the sequence of the gC gene optimized by substitution, by a signal sequence, in particular that of the tPA signal of human origin, of the sequence of the signal peptide of the gC glycoprotein, and/or by the deletion of the DNA fragment encoding the transmembrane domain of gC.

33. Vaccine or immunogenic or immunological composition according to Paragraph 30, wherein it comprises the sequence of the gD gene optimized by substitution, by a

signal sequence, in particular that of the tPA signal of human origin, of the sequence of the signal peptide of the gD glycoprotein, and/or by the deletion of the DNA fragment encoding the transmembrane domain of gD.

34. Vaccine or immunogenic or immunological composition according to Paragraph 30, wherein it comprises DMRIE-DOPE, an expression plasmid encoding the gB antigen of PRV optimized by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain and of the contiguous C-terminal part, a second expression plasmid encoding the gC antigen of PRV optimized by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain and of the contiguous C-terminal part, and a third expression plasmid encoding the gD antigen of PRV optimized by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain and of the contiguous C-terminal part.

35. Vaccine or immunogenic or immunological composition according to Paragraph 1, wherein it comprises a nucleotide sequence of PRRSV.

36. Vaccine or immunogenic or immunological composition according to Paragraph 35, wherein it comprises a nucleotide sequence of the ORF3 gene optimized by substitution, by a signal sequence, in particular that of the tPA signal of human origin, or the sequence of the signal peptide of the protein encoded by ORF3, and/or by the deletion of the DNA fragment encoding the transmembrane domain of ORF3.

37. Vaccine or immunogenic or immunological composition according to Paragraph 35, wherein it comprises a nucleotide sequence of the ORF5 gene optimized by substitution, by a signal sequence, in particular that of

the tPA signal of human origin, or the sequence of the signal peptide of the protein encoded by ORF5, and/or by the deletion of the DNA fragment encoding the transmembrane domain of ORF5.

38. Vaccine or immunogenic or immunological composition according to Paragraph 35, wherein it comprises a nucleotide sequence of the ORF6 gene optimized by substitution, by a signal sequence, in particular that of the tPA signal of human origin, or the sequence of the signal peptide of the protein encoded by ORF6, and/or by the deletion of the DNA fragment encoding the transmembrane domain of ORF6.

39. Vaccine or immunogenic or immunological composition according to Paragraph 35, wherein it comprises DMRIE-DOPE, an expression plasmid encoding the ORF3 antigen of PRRSV, a second expression plasmid encoding the ORF5 antigen of PRRSV optimized by substitution of the signal sequence of ORF5 by the human tPA signal peptide sequence and by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain and the contiguous C-terminal part, and a third expression plasmid encoding the ORF6 antigen of PRRSV optimized by the substitution of the signal sequence of ORF6 by the human tPA signal peptide sequence and by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain and the contiguous C-terminal part.

40. Vaccine or immunogenic or immunological composition according to Paragraph 1, wherein it comprises a nucleotide sequence of SIV.

41. Vaccine or immunogenic or immunological composition according to Paragraph 40, wherein it comprises a nucleotide sequence of the HA gene optimized by

substitution, by a signal sequence, in particular that of the tPA of human origin, of the signal sequence of HA, and/or by the deletion of the DNA fragment encoding the transmembrane domain of HA, and/or by the insertion of an intron, in particular of intron II of the rabbit beta-globin gene upstream of the nucleotide sequence encoding HA.

42. Vaccine or immunogenic or immunological composition according to Paragraph 40, wherein it comprises a nucleotide sequence of the NA gene optimized by substitution, by a signal sequence, in particular that of the tPA of human origin, of the signal sequence of NA, and/or by the deletion of the DNA fragment encoding the transmembrane domain of NA, and/or by the insertion of an intron, in particular of intron II of the rabbit beta-globin gene upstream of the nucleotide sequence encoding NA.

43. Vaccine or immunogenic or immunological composition according to Paragraph 40, wherein it comprises DMRIE-DOPE, an expression plasmid encoding the HA antigen of SIV optimized by the insertion of the signal sequence of the human tPA in place of the signal sequence of HA, by the deletion of the fragment of the nucleotide sequence of HA encoding the transmembrane domain and the contiguous C-terminal part, and by the insertion of intron II of the rabbit beta-globin gene upstream of HA, and a second expression plasmid encoding the NA antigen of SIV optimized by the insertion of the signal sequence of the human tPA in place of the signal sequence of NA, by the deletion of the fragment of the nucleotide sequence encoding the transmembrane domain of NA and the contiguous C-terminal

part, and by the insertion of intron II of the rabbit beta-globin gene upstream of NA.

44. Vaccine or immunogenic or immunological composition according to one of paragraphs 9 to 43, wherein it also comprises DOPE.

45. Vaccine or immunogenic or immunological composition according to one of paragraphs 9 to 43, wherein it comprises, in addition, a GM-CSF protein of the animal species considered.

46. Vaccine or immunogenic or immunological composition according to one of paragraphs 9 to 43, wherein it comprises, in addition, an expression vector containing the gene encoding the GM-CSF protein of the animal species considered, under conditions allowing the *in vivo* expression of this sequence.

47. Vaccine or immunogenic or immunological composition according to one of paragraphs 9 to 43, wherein the expression vector is a plasmid.

* * *

It should be clearly understood that the invention defined by the appended claims is not limited to the specific embodiments indicated in the description above, but encompasses the variants which depart neither from the scope nor the spirit of the present invention.